STUDIES ON THE TECHNIQUE OF SKIN TESTING IN ALLERGY

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W. J. F. VAN DER BIJL

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS MR. J. E. JONKERS, HOOGLERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER GENEESKUNDE TE VERDEDIGEN OP WOENSDAG 9 DECEMBER 1959 TE 16 UUR

DOOR

WILLEM JAN FREDERIK VAN DER BIJL
geboren te Arnemuiden in 1910

H. E. STENFERT KROESE N.V. — LEIDEN

PROMOTOR: PROF. DR. H. A. E. VAN DISHOECK

Aan de nagedachtenis
van mijn ouders en mijn broer

Dit proefschrift werd bewerkt in de afdeling voor Keel-, Neus- en Oorheelkunde van het Academisch Ziekenhuis te Leiden en in de Amsterdamsche Kliniek voor Allergische Ziekten.

Allergeenextracten werden bereid in het laboratorium van de Amsterdamsche Kliniek voor Allergische Ziekten en bij de Lifa te Groningen.

Oplossingen met een bepaalde P_H werden bereid in de Apotheek van het Academisch Ziekenhuis te Leiden.

De dierproef werd mogelijk gemaakt door het Hoofd van het Bacteriologisch Laboratorium van de G.G. en G.D. te Amsterdam. De overdrukinstallatie voor het verhitten op zeer hoge temperaturen, verschafte mij het Fysiologisch-Chemisch Laboratorium van de Vrije Universiteit te Amsterdam.

Festina lente

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CONCLUSIES

1) Van de cutane huidreacties heeft de scratchtest de voorkeur boven de prik- en de boorproef, omdat de eerste verreweg het minst onaangenaam is voor de patiënt.

2) Huidreacties moeten worden beoordeeld t.o.v. reacties met histamine en een controle met de extractvloeistof. Aan de histamine reactie 1/10.000 moet +++ worden toegekend, bij intracutane huidproeven dient 0.02 cc te worden ingespoten. Pseudopodiën worden met ++ extra gehonoreerd.

3) Bij het reinigen van spuiten en naalden is de mechanische reiniging belangrijk. Vóór het steriliseren, dient het met allergenen besmette materiaal eerst gedurende 5 min. te worden gekookt, waarna, na uitlekken, in een andere pan sterilisatie volgt. Koken dient niet in horizontale stand te geschieden.
Het materiaal voor het allergenen-onderzoek en dat voor therapeutische doeleinden moet streng gescheiden blijven.

4) Voor het verrichten van huidproeven dient men de huid te reinigen met aether, dat na 2 minuten geen invloed meer op de grootte van de huidreacties heeft. Adrenaline beïnvloedt het resultaat niet meer na 1 uur; de werking van Thephorin is wisselend, het gebruik hiervan dient, evenals voor alle antihistaminica geldt, te worden ontraden kort voor een huidproef wordt verricht.

5) Het blijkt dat alle allergenen thermostabiel zijn, dat langdurig koken de allergeenpotentie na 2 uur vermindert, dat dit bij 180° een half uur duurt en dat ze zeer hoge temperaturen (300°) niet verdragen.
Chemische sterilisatie met Coca's vloeistof of ½% Phenol beinvloedt de huidreactie weinig; bij filtratie treedt allergeenverlies op, vooral door een Seitzfilter.

6) Chemische sterilisatie remt gemiddeld 10 weken bacteriegroei in de allergeenextracten. Hiermede dient men vooral bij desensibilisatie vloeistoffen rekening te houden. De pH van de extractie-vloeistof is van weinig belang, zowel wat betreft bacteriegroei, als voor de potentie van de extracten.

7) Rationalisatie van het allergenen-onderzoek door groepering van allergenen is practisch. Doorgroeperen tot een mengsel van alles, een zgn. „screen" is vooral belangrijk ter voorkoming van het risico van shock bij het routine-onderzoek. Samen met groepen allergeenextracten krijgt men met 11 injecties een vrij goed inzicht of een patiënt tot de atopische groep behoort.

8) Het blijkt, dat de inhalatie-allergenen het meest betrouwbaar zijn, dat van de voedingsmiddelen vooral de groenten en fruitsoorten ons nogal eens in de steek laten. Behalve zgn. „propagatieve stoffen" spelen naast dag- en seizoensinvloeden nog andere onbekende factoren een rol.

9) Voor experimenteel onderzoek dient men gebruik te maken van zelfgemaakte verse extracten, voor het routine-onderzoek zijn commerciële extracten goed bruikbaar.

10) De Prausnitz-Küstner reactie is vooral voor kwantitatief onderzoek belangrijk, mits men daarbij van een compressor gebruik maakt.

INTRODUCTION

The group of specific sensitivities designated as "allergy" occupy a special place among immunological manifestations.

In infectious diseases, the toxins produce antibodies resulting in immunity, whereas in allergy these antibodies, so-called reagins, are the very cause of the clinical symptoms.

The fact that these reagins or antibodies are demonstrable is a valuable aid in clinical diagnosis. For it is an extremely curious finding that patients will develop an urticarial wheal immediately after pollens have been rubted into a cut made into the skin, a reaction that undoubtedly ranks among the important medical phenomena.

In addition to these immediate reactions, there also are delayed reactions such as those accurring in tuberculin tests and the skin reactions appearing when a chemical is applied to the skin. Immediate reactions are assumed to be mainly associated with reagins in the blood and vascular lesions (Hansen [4]), whereas delayed reactions are believed to be associated with reagins attached to the cells and tissue changes (Jiménez Díaz and Arjona [25]). So far, little is known about these delayed reactions. It has not been possible to demonstrate a relationship between serum antibodies and those of cells. The two can only be shown to be present by specific reactions.

Of the many hypotheses suggested to explain the mechanism of the two types of reaction, Raffel's theory [7] is the most attractive. It is based on experimental studies by Dienes [7] who induced a delayed reaction by injecting a non-bacterial agent, viz. egg albumin, into a tuberculous lesion in a guinea pig. Raffel found both egg albumin and tuberculoprotein to produce allergic reactions of the immediate type in guinea pigs. Intraperitoneal administration of egg albumin and a purified "wax fraction" of the tubercle bacillus produced a delayed reaction. Raffel's theory was that a wax-like substance, to which the appearance of this delayed form of hypersensitiveness is attributable, is essential to the appearance of delayed reactions. Mayer [8] made further additions to Raffel's theory, claiming that liquid non-oriented globular proteins produce immediate reactions, whereas delayed reactions are due to oriented (fibroid) proteins, cell constituents (Raffel's wax fraction) or micro-organism.

Accordingly, the route by which the antigens enter the body will depend on particular conditions.

The antibodies causing delayed reactions have not been identified. They are not passively transferable with serum, though passive transfer

is successful with living or disrupted cells of the leucocyte group of the allergic organism.

Immediate reactions include the following types of reaction:

a) anaphylactic shock;

b) so-called atopic reactions which include serum sickness, asthma, pollinosis, angio-oedema, periarteritis nodosa (possibly also glomerulonephritis, rheumatic fever and rheumatoid arthritis).

Delayed reactions include:

reactions to parasites (flea-bite), contact with chemicals, primroses, etc.

The artificially induced skin reactions include:

a) immediate reactions:
in the scratch, prick, "bore" and intracutaneous tests;

b) delayed reactions:
in the scratch test (Pirquet's test), the intracutaneous test and the patch test.

In addition to these skin tests, nasal, pulmonary and conjunctival mucous membrane tests are used as well. These are the so-called provocation tests. The methods used in making these tests also vary widely.

Opinions differ regarding the skin tests which are to be preferred as well as the method to be used in testing.

Some believe that the skin should not be cleaned with alcohol or ether, as this might adversely affect the size of the skin reactions. There also is considerable misconception concerning the sterility and stability of allergenic extracts. Some apply the allergens in the dry state, whereas other only use solutions. As a rule, insufficient attention is paid to the requirements to be met by the allergens.

Opinions also differ as to the methods to be used in sterilizing syringes and needles and in freeing them from allergens.

The tests elaborated in greatest detail are those with chemicals in which reactions are produced by sticking a small piece of cloth on to the skin. This is due to the fact that, unlike the allergens, the substances used in this case are known chemicals which therefore may be readily standardized on normal individuals. It is much more difficult to standardize inhalants and food allergens which may cause asthma and diseases of the nose.

For all these reasons, the present author set himself the task of studying the methods to be used in greater detail.

Opinions on the diagnostic value of skin tests vary. Allergists frequently tend to overrate these tests, whereas they are underrated by others. Skin tests undoubtedly provide conclusive evidence in conditions such as hay fever and various forms of occupational allergy. This is not always the case in other forms of allergic disease. The fact that sensitivity

is present may, however, be established by these tests. Accordingly, an asthmatic patient showing a positive skin reaction to a particular food need not necessarily have an attack of asthma on ingestion of this food. He may, however, have an attack of urticaria in particular conditions.

Peshkin [34], and Van Dishoeck [19] differentiate between the following contingencies:

1) The positive reaction is indicative of the causative factor at this stage in the course of the disease.
2) In addition to other aetiological factors, the allergen producing a positive reaction is a part of the allergic syndrome present at the time.
3) The positive reaction is evidence of a previously present aetiological factor which may become manifest if the exposure to the allergen is sufficiently intensive and sufficiently prolonged.
4) The allergen is not one of the factors that have caused the disease, though it may subsequently affect its course or give rise to complications.

Duke, Pasteur-Valery Radot and Blamoutier as well as Storm van Leeuwen [6] draw attention to the fact that it often is difficult to interpret the skin reactions. Only the result obtained in treatment will show whether the allergic disease actually was due to a particular allergen. Moreover, it should be borne in mind that, despite these doubts, skin tests frequently provide the only basis for treatment by elimination or desensitization. Therefore, though the skin reactions cannot remove all doubt, they will, as has been stated previously by authors such as Storm van Leeuwen and Kremer [27], provide valuable data on the allergic condition of the entire organism in a large number of cases.

Skin reactions and sensitivity are undoubtedly associated in pollen allergy. Accordingly, the most satisfactory results are obtained in the treatment of pollinosis.

Alexander [13] found tests using inhalants to be much more reliable than those with food allergens in about 11,000 cases. The proportion of correct findings was 93.2 per cent in patients with pollinosis and 26.5 per cent in cases of gastro-intestinal allergy.

When the correct method of testing and the correct method of preparing allergenic extracts have eventually been established, skin tests will be immunologically specific (Tuft) [41]. Until then, results should be critically evalutaed and the Prausnitz-Küstner test resorted to if necessary.

Skin reactions will vary with:

1) The *site* of injection: the back shows a better response than do the arm or the thigh.

2) The *potency* of the allergens: this will depend on the nature and age of the extract and on the material used in extracting.
3) The *circulation* within the skin: the skin reaction may be reduced or become negative in peripheral vascular disturbances. Local anaesthetics or cooling are assumed to have an adverse effect on skin reactions. In addition, the circulation may vary with seasonal changes.
4) The *age* of the patient: elderly subjects are less liable to develop erythema than are young subjects. Whealing occurs to an equal extent in young and old subjects. Some even believe that the wheals are more marked in elderly subjects.
   The number of substances to which a patient reacts will increase with the age of the subject (Brutel de la Rivière [16]).
5) The *race* of the patient: Thommen (Vaughan[11]) found that pure races usually are less frequently affected with allergy in the districts where they have their home than they are when living as strangers in another district.
6) The *condition* of the patient: what did he eat shortly before testing? Did he take any drugs shortly before? Are relationship reactions likely to occur? (For instance, a positive reaction to horse dander may occur when the patient has previously been given horse serum).
7) Have there been any *technical errors:*
   a) has the lesion been too severe?
   b) have the sites of pricking or cutting been placed to close to one another?
   c) has there been too early or too late reading?
   d) has there been any infection with other allergens?
8) Has any *technical error* been committed in *preparing* the allergen? Does the preservative act as an irritant?
9) Is there a *refractory period?* Some authors believe the reactivity of the skin to diminish shortly after an attack of allergy; this is denied by others.
10) Are there *blocking antibodies?:* skin reactions become less marked following prolonged desensitization; they even may become completely negative when an antibody directed against the reagins is produced.
11) Gastric and intestinal juices may convert a food into a product of digestion, causing a great or lesser degree of allergy.

The very young special department of medicine referred to as allergology, like any young state, is still in the revolutionary stage. This implies that everyone has his own method of dealing with allergy. The serologist, for instance, will use methods that differ from those of the clinician; in particular, specialists in diseases of the ear, nose and throat, specialists

in internal medicine, chest physicians and dermatologists, each in his own field, frequently employ entirely different methods of skin testing. There also are a number of general practitioners who are interested in the subject. The second world war has been followed by some grouping and associating both on a national and on an international scale, but this has certainly not reduced the dissension and mutual criticism among the various groups. A number of the above problems relating to methods of testing in allergy will be studied in greater detail in the following chapters:

Chapter I is concerned with a review of the techniques of skin tests producing reactions of the immediate and delayed types. The advantages and disadvantages of the scratch, "bore", prick and intracutaneous tests are weighed against each other.

Chapter II deals with the interpretation and recording of the results obtained in skin testing.

Chapter III is concerned with freeing the syringes and needles of allergens.

Chapter IV is concerned with a number of observations and considerations on the reactivity of the skin, e.g. that associated with variations of drugs, disinfection, daily and seasonal changes.

In *Chapter V*, the Prausnitz-Küstner test is discussed as a method used in skin testing in allergy.

In *Chapter VI*, the preparation and preservation of sterilized allergenic extracts is discussed in greater detail in view of the importance of testing to allergens in medical practice.

Chapter VII is concerned with the question of the extent to which testing to allergens may be reduced to a few cutaneous or intracutaneous tests, which might show whether or not a patient is allergic. In addition, efforts are made to describe the safest, simplest and most reliable method that may be used to obtain the most satisfactory results with the smallest risk of systemic reactions. The reactions produced by personal allergenic extracts are compared with those produced by a number of commercial extracts.

The extent to which skin tests may be of value in establishing the diagnosis, is not considered in the following chapters.

CHAPTER I

THE TECHNIQUE OF SKIN TESTING

A) IMMEDIATE REACTIONS IN SKIN TESTING

The skin is the organ most readily accessible to the eye and therefore best suited for determining the response of a patient to substances foreign to the body. Attempts to do so go back as far as the latter half of the nineteenth century. Since Von Pirquet [1] described his tuberculin skin test in 1907, skin tests have become a valuable diagnostic aid, especially on the initiative of Storm van Leeuwen and his associates.

Skin tests, however, were soon found not only to offer advantages, but also to be attended by hazards.

Efforts were made to obtain the clearest possible reactions, but it was observed that clear and frequent reactions on the one hand were difficult to combine with safety on the other.

Within a year after von Pirquet's tests had been introduced, methods of skin testing were split up into a scratch test and an intracutaneous test. The former is safer than the latter. Among the advantages of the scratch test is the fact that syringes and needles are not required and that only a smal quantity of reagin is introduced into the skin. This reduces the risk of systemic reactions. As a rule, a fair number of tests may be performed simultaneously and, provided a different vaccinostyle is used in each patient, there will be no danger of infection and homologous serum jaundice. Accordingly, there are few problems of sterilization and dry allergens remaining stable over prolonged periods may be used.

Among the drawbacks are the fact that the concentration of the allergens is low, so that the results obtained in testing will not be sufficiently reliable, that scarification with a vaccinostyle causes a relatively large lesion, so that non-specific side-effects will be more liable to occur in subjects showing marked dermographism. In the course of time, a number of modifications of this scratch test have been suggested with a view to removing some of these disadvantages; a relative degree of insensitiveness will, however, continue to be present in any form of skin testing.

The intracutaneous test, on the other hand, is a hundred times more sensitive than the cutaneous test. This method of testing, however, involves the use of a fairly large number of syringes and sterile material. In addition, allergenic extracts remaining stable for a limited period are used and there is an increased risk of systemic reactions.

It is advisable:

1) to start with a cutaneous test, because it will take more time to overcome the physiological barrier, as the allergen is absorbed more slowly;
2) to avoid performing skin tests in patients with conditions such as pollinosis during the hay fever season, as the sensitivity of these subjects can be expected to increase during this period.

The other advantages and disadvantages of the various methods of skin testing will be discussed again in reviewing the technique of testing.

§ 1) *Cutaneous testing*
A 1) The scratch test
A cut is made into the skin with a vaccinostyle, needle or scalpel without drawing blood.

The cut should be made parallel to the axis of the arm in the forearm or, if done on the back, parallel to the spinal column. The cuts should preferably be 0.5 cm. in length and placed at least 2.5 cm. apart. An interval of 4 cm. is the most suitable. The extract of the allergen is then applied to the cut with a sterilized glass rod or toothpick and gently rubbed into it, or a drop of 0.05 of 0.1 N sodium hydroxide is applied to the cut, the precipitated dry rest of the extract or the allergen in its initial state, whether or not defatted, then being rubbed into the drop. Cooke [1] points out that the material should not be allowed to dry up before the results have been read. Conceivably, the reagins might be absorbed more slowly on desiccation or when dry allergens are applied.

A 2) The 'bore' test
The 'bore' test is a modification of the scratch test in which the cut into the skin has been replaced by a lesion made by a simple, superficial act of boring, the instrument being rotated through an arc of 90^0.The small chisel, which is rotated between thumb and index, is about 4 cm. in length and half of the upper part has been made rough to prevent the fingers from slipping; the sharp lower part is 2 mm. wide.

B 1) The prick test
The prick test, which was introduced by Lewis and Grant [29], has been mainly adopted in Great Britain and to a lesser extent in Sweden and the U.S.A. A drop of allergenic extract is applied to the skin with a rod. The prick test instrument is then pricked once through the drop into the skin, so that it hurts a little, though without drawing any blood. The instrument should be thoroughly wiped and cleaned after the prick has been made, so as to prevent the contamination of allergens.

Squire [38] believes this procedure to be even less hazardous than the scratch test. The lesion produced is very slight in this case.

B 2) There is a *modification* of this test in which a prick is not made quickly through the drop, but the tip of the instrument is placed in the drop and pressed gently upon the skin until it is just beneath the latter. The instrument is then rapidly placed in the vertical position and withdrawn. This might be called a method intermediate between the prick test and the intracutaneous test; it is especially recommended for use in children.

Once a sufficiently positive reaction has developed in these skin tests, the allergen may be wiped off with a piece of cotton-wool impregnated with alcohol, which prevents further absorption. Depending on the history, the age of the patient and the potency of the allergens, not more than 25 tests should be performed at each sitting. This number should not be exceeded, as otherwise the risk of a systemic reaction will be anything but imaginary.

Cutaneous testing is commendable in view of its simplicity, though there are several drawbacks to this method.

The chief drawback is that an unknown quantity of reagin is absorbed. Moreover, nearly every investigator uses his own technique, so that it is difficult to compare the results obtained.

Skill is required to avoid abrading or pricking beyond the outer dead layer of the skin and to avoid penetrating too far into the live epidermis. Too severe a lesion of the skin will frequently produce a considerable mechanical stimulus and inconvenient bleeding.

Severe systemic reactions are hardly ever observed when these tests are performed. Only *three* sudden deaths have been reported in the literature, two following a skin test with an extract of buckwheat flour and one following a skin test with an egg extract (Vaughan [11]). Severe, though not fatal reactions have been reported as following scratch tests with extracts of chocolate, pea-nuts and pollen.

Personal investigations

Comparative experimental studies on the scratch, 'bore' and prick tests
In order to acquire a better knowledge of the advantages and disadvantages of these various methods, the reactions to the test with the vaccino-style (A), the modified prick test (B) and the 'bore' test (C) were compared in 71 subjects who had been out-patients for some time previously. They all were allergic to house dust and fungi, a fact determined several times. The tests were made using a mixture of the two extracts $\overline{aa}$-1 per cent.

All skin tests were performed by the same assistant under the supervision of the present author, who read the results within exactly 20 minutes after the lesion had been made.

In addition, it was noted which of the three tests was the most unpleasant to the patient.

In children under 12 years of age, the comparative tests were performed after a number of scratch tests with allergens had been previously made at this sitting.

This precaution was adopted to ensure that the information on the painfulness or non-painfulness of the pricks and cuts should be as reliable as possible (cf. Table I).

The skin reactions were evaluated in relation to 1 : 10.000 histamine and a control, as described in Chapter II (p. 16). To enable a comparison of the results, a + sign was reckoned as equal to 1, a ± sign as equal to ½ and a — sign as equal to 0. In the last column a letter has been used to indicate which of the three tests was the most unpleasant to the patient. The absence of a letter signifies that the patient did not perceive any difference in the intensity of the pain.

The scratch test scored 34 points, the 'bore' test 32. Therefore, there is no difference in favour of either test from the clinical point of view. As the 'bore' test was stated to have been unpleasant in 13 cases, however, and the scratch test was only stated to have been unpleasant in 2 cases, the conclusion would seem to be justified that the latter is the better test.

The results obtained in the modified prick test were markedly inferior to those obtained in the two other tests.

Conclusion: the scratch test with the vaccinostyle is the best epidermal skin test as compared with the 'bore' test and the modified prick test.

§ 2) *Intracutaneous testing*

After Mantoux and Mendel [17] had described the intracutaneous tuberculin test independently of each other in 1908, Schloss introduced this test with allergens in 1912.

A glass tuberculin syringe or a finely graduated 0.5 or 1 ml. record syringe are employed in this method of testing.

The needle should be short (1—2 cm. in length) and have a short bevel. From 16 to 22 gauge needles may be used for this purpose.

After the liquid to be injected has been drawn into the syringe, all air bubbles should be removed, as even the injection of minute quantities of air into the skin may result in marked false positive reactions.

It is essential that the needle should be inserted superficially and that the injection should be made *into* the skin and not just through it, the bevel being directed upwards. The syringe should not be rotated in doing so; this would both increase the lesion and might possibly cause leakage.

Only a very small quantity of allergen should be injected; as little as 0.02 ml. will be sufficient (cf. p. 17). Initially, 0.1 ml. was injected; this too large quantity did not only increase the risk, however, but also frequently caused non-specific reactions.

TABLE I

Case	m/f	age	diag-nosis	A	B	C	pain most intense in	Case	m/f	age	diag-nosis	A	B	C	pain most intense in
1	f.	28	U	+	±	—	C	26	m	23	Pol.	—	—	±	
2	m	60	A	+	—	±	C	27	m	18	A	±	±	—	
3	f	5	E	±	+	+	C	28	m	53	A	+	+	+	
4	m	25	V.rh.	+	±	+	C	29	m	59	U	—	+	—	C
5	f	50	A	+	—	+		30	f	58	U	—	+	—	
6	f	48	U	+	+	+	C	31	f	14	A	+	+	+	
7	f	26	V.rh.	±	—	±		32	f	30	U	—	—	±	C
8	f	6	U	±	+	—	B	33	f	11	E	±	—	±	
9	f	8	A	+	+	±		34	f	63	Ang. oed.	±	—	±	
10	f	20	V.rh.	+	+	+									
11	m	30	A	+	—	±	C	35	m	14	V.rh.	±	—	±	
12	m	20	A	—	—	++	C	36	m	37	E	+	+	—	
13	f	72	A	±	—	—	A	37	m	57	A	+±	±	±	
14	m	3	U	+	+	+		38	f	6	A	±	±	±	
15	m	25	A	+	+	+		39	f	31	V.rh.	+	—	+	
16	m	23	A	±	±	±		40	m	64	A	±	—	+	
17	f	29	Pol.	±	—	±	C	41	f	25	A	—	+	±	C
18	m	58	A	+	—	—		42	f	14	V.rh.	±	—	±	BC
19	m	7	A	+	±	++		43	f	33	V.rh.	±	—	±	
20	f	59	Ang. oed.	±	±	—		44	f	57	A	+	—	—	
								45	m	11	V.rh.	±	—	—	
21	m	22	Pol.	++	±	+		46	f	56	V.rh.	++	+±	++	AB
22	f	64	A	±	—	+±	C	47	f	39	A	±	—	—	
23	f	44	A	+	—	±		48	m	67	A	±	—	±	
24	m	32	E	—	—	+		49	f	51	A	—	—	±	
25	m	44	V.rh.	±	±	+		50	m	49	V.rh.	±	—	—	

	34	19½	32

Pain most intense in:	A	B	C
	2	3	13

As soon as the injection is started, the skin of the arm is stretched by taking hold of its posterior aspect with the free hand. The amount of allergenic extract injected should be exactly the same in every injection; both the arm of the patient and that of the physician should be sufficiently fixed.

The intervals between the sites of injection should be 4 cm. A different syringe and needle should be used in testing each allergen.

The extremities are to be preferred in using this method, as the flowing off of blood and lymph may be interrupted by ligation in the event of an imminent systemic reaction, so that further absorption will be prevented. The tests may only be made upon the back when it has been definitely established that the patient is not particularly sensitive to the allergens to be tested.

Depending on the history of the patient, it is advisable not to make more than 18 injections at each sitting.

Storm van Leeuwen was among the first authors to sound a note of warning against the hazard of systemic reactions. He advised diluting the extracts to a considerable degree if necessary. When marked sensitivity is suspected, the precaution of starting with an intracutaneous injection of a 1 : 100 or 1 : 1.000 dilution of the allergen or of starting with the cutaneous test should be taken.

Though Walker [6] warns against the fact that intracutaneous injections are liable to produce non-specific false reactions, Brown [15] and several others regard this method as being more reliable than the scratch test.

A serious drawback to the intracutaneous test is that a large number of syringes will have to be available to enable a complete examination. Therefore, the question was asked whether it might be possible to obtain sufficient data with a smaller number of tests by using mixtures of allergens. The results obtained in tests of this type will be reported in Chapter VII.

B) DELAYED REACTIONS IN SKIN TESTING

The epidermal patch test

The patch test is a valuable diagnostic aid in dermatological manifestations of allergy. In this method of testing, recommended by Jadassohn and subsequently elaborated by Bloch and his assistants the allergen is applied to a normal area of the skin in the dry state or in the form of a paste.

As a rule, the Schwartz standard patch is used, consisting of a piece of flannel, about 2 sq.cm., which is moistened with 0.2 ml. of sterile water prior to applying from 0.05 to 0.2 ml. of the substance to be tested to the flannel with a pipette. This is applied to the skin and then covered with an insulating layer such as Billroth's cambric (synthetic substances

should be avoided!). Over this there is applied an adhesive plaster which is attached to the skin.

When the patient is known to be sensitive to adhesive, a piece of muslin attached by mastisol and held in place by a muslin dressing should be used for insulation.

The dressing or adhesive is removed 24 hours later and the area of skin is cleaned, undue irritation of the skin being avoided.

Before reading the reaction, it is advisable to wait for 15 to 30 minutes until any redness caused by compression will have disappeared. In interpreting patch tests, it often is very difficult to differentiate between primary irritation and allergy. The area of irritation usually disappears rapidly on removal of the material, the allergic reaction frequently becoming more pronounced within from 1 to 2 days. Pruritus is a fairly common symptom in positive tests and persists for a longer period than does ordinary irritation.

It will be necessary to modify this technique in some cases. For instance, in testing for sensitivity to certain parfumes, it will be advisable to approximate actual conditions of contact as closely as possible. The so-called open patch test, rather than the closed patch test previously described, is used in these cases. The material to be tested is applied to sensitive areas of the skin such as the popliteal space, the folds of the elbow and the skin behind the ears. The substance is wiped off within 24 hours and the reaction is read; in the event of a negative reaction, the test is repeated, even on three consecutive days if necessary. When the reaction continues to be negative, sensitivity is assumed to be absent.

There is a wide range of variations between these two extremes as represented by the open and closed patch tests; these variations are designed to avoid complete insulation of the material, as total occlusion may cause an inoffensive substance or solvent, which usually evaporates when applied to the skin, to act as an irritant.

Another variation consists in the occasional performance of a so-called scarified patch test with a view to increasing the reaction. When the direct test with the material to be tested is found to be negative after 20 minutes, the patch is applied to the scarified area.

Patch tests differ from other skin tests in that the skin continues to have a protective action in the former, the reaction being epidermal; in addition, the material is absorbed less rapidly than it is in intracutaneous tests in which the corium is closely approached. Therefore, hardly any changes are detectable in the upper layers of the corium on microscopical examination in the patch test, whereas there are slight degenerative changes of the epidermal cells; it is very difficult, however, to determine the nature of these changes.

The reaction to the substance is an eczematous reaction in the patch test, intracutaneous tests producing a wheal or urticaria.

It is essential that the substance to be tested should not be toxic or applied in concentrations having irritating or toxic effects. Therefore ointments may be used, into which the material to be tested has been incorporated. An ointment will not dry up and will remain exactly on the areas of the skin to which it has been applied, so that the allergen may exert its action at this site.

Rostenberg [6], Sulzberger [6], Herff [5], Schwartz [9], Tulipan [9], Birmingham [9] and others have compiled lists showing the concentrations required in this method of testing. Others have pointed out, however, that in addition to the fact that these lists are incomplete owing to the large number of substances which may act as allergenic compounds, it also is doubtful whether these concentrations are actually reliable.

On the other hand, an ointment base itself may cause an allergic skin reaction or eczema. Particularly in cases of occupational and drug allergy, it is of vital importance to know *which* concentration of the chemical will just fail to produce non-specific irritation. Nor should the contentration be too low, however, to prevent the substance from producing a positive reaction. The composition of the contactants or chemicals is of special importance in tests for the action of chemical compounds, i.e. in occupational diseases and in individuals using cosmetics. For some of these compounds may occasionally be non-specific primary skin irritants and, moreover, these skin irritants frequently have a sensitizing effect which is more marked than that of substances which do not cause non-specific irritation.

Strong acids or alkalis will always be primary irritants and burn every skin.

In special cases, a control patch test on a normal volunteer will be advisable.

A positive reaction is expressed in terms of degrees. The following classification is used:

1st degree: redness;
2nd degree: redness, swelling and incipient whealing;
3rd degree: redness, swelling, marked whealing and petechial haemorrhage;
4th degree: redness and inflammatory symptoms, vesiculation and incipient superficial necrosis;
5th degree: local epidermolysis and, to some extent, spreading of the symptoms to the surrounding skin.

A positive reaction merely means that a *particular* area of the skin is sensitive to *that* substance at *that* time. When there is a negative reaction, then it does not always follow that there is no allergy to this substance, as the fact that a reaction is positive or negative will depend on:

1) the concentration of the material applied;
2) the quantity (and composition) of the substances to be tested;

3) the site where the test is performed;
4) the size of the patch test;
5) the time of reading and, especially, the time that has elapsed between the removal of the adhesive and the reading;
6) the presence or absence of primary irritants;
7) the place where the test is performed.

Nature cannot be reproduced in performing the patch test. The results may be affected by heat, cold, sunlight and additional factors related to the microclimate of the skin.

The skin may not be sensitive, not yet sensitive or less sensitive locally. The tough, hard skin of the hands will react less intensively than that of the forearm or that behind the ears. An atrophic skin will also show a reduced degree of reactivity. It may only be hypersensitive during eruption.

The degree of acidity of the skin may be of importance in the closed patch test and definitely is an important factor in the open patch test. The pH is more alkaline in the axilla than it is in other areas of the skin. Marked pigmentation is also believed to reduce sensitivity.

Opinions differ regarding the site of the skin on which the test should be performed. Some prefer the back, others the thigh, though it is advisable to make the test on the normal skin near the area of the eczema, as sensitization decreases from the site of contact outward. Some believe the normal areas of the skin of a patient with eczema to be seven times as sensitive as those in normal subjects. It is not stated, however, whether this increased sensitivity is only present in the acute stage. This acute stage might also be due to secondary infection.

Allergy resulting in a negative reaction may occasionally be observed in the acute stage. Summation may sometimes be necessary to produce a positive reaction. This has been reported as occurring in the case of certain cosmetics.

In addition, a positive reaction may appear several years later at a site where the results obtained in repeated patch tests were negative. This may be due to an improvement technique or to sensitization, or might it be possible after all that allergens are capable of spreading from one skin cell to another? Finally, it should be borne in mind that a patient with eczema need not be merely allergic to chemicals, but may also show an endogenous, haematogenous allergy in addition to this exogenous form of allergy.

The simplicity of the patch test and the fact that absorption of the material is slow do not mean that complications cannot occur. Not only have there been reports of systemic reactions which were even associated with rises in temperature and lymphadenopathy, but there may occasionally be a recurrence of a healed dermatitis or a flare-up of a healed patch test site. Keloids may also occur in some cases. Therefore, it is advisable

to confine testing to *those* substances with which the patient comes into contact and/or those which are suspected in his case. In view of the risk of sensitization, the material should not be allowed to remain in place for a period beyond 5 days in testing for diagnostic purposes.

Opinions also differ on the diagnostic value of the patch test. Odegaard sets great store by this test, stating that it only fails in 17 per cent of the cases. The patch test undoubtedly is a valuable diagnostic aid, however, and should be used to establish the diagnosis, in conjunction with the history, the study of the clinical symptoms and examination of the tissues by punch biopsy.

The above shows that interpretations should be very critical and that it is precisely because it is so difficult that interpreting the tests is a specialist's job (Mali [30]).

CHAPTER II

INTERPRETING SKIN REACTIONS

In order to be able to decide whether a skin reaction may be considered positive, the possibility of non-specific irritation should be ruled out. Therefore, a control injection of the extracting fluid and diluent should be made in every case. In addition, the reactivity of the skin should be tested with 1 : 10.000 histamine. The first control injection should be negative and the last injection should be positive.

Gay and Chant (1927) observed that both traumatic and non-specific reactions are most marked after 15 minutes and that they disappear during the next 15 minutes, whereas specific reactions continue to be perceptible for a much longer period. Klein also observed this phenomenon. Therefore, these authors suggest interpreting the reaction again after 30 minutes. It may occasionally be necessary to request the patient to return a few hours or days later, to see whether there have been any delayed reactions.

A skin test is designated as positive when a wheal surrounded by erythema has occurred. The wheal is the chief criterion in interpreting the reaction. The erythema merely is evidence of the reflexmechanism and disappears on elimination of the nerve supply (Urbach [17]). The appearance of pseudopods is a particularly important feature in wheals.

In classifying reactions, not only the size and shape of the lesions, but also the extent to which they are elevated above the surface of the skin should be taken into account, the consistence of the wheal being determined by palpation. Accordingly, reading skin reactions calls for self-criticism as well as experience.

The clinical data, the age of the patient (the skin of a child will show a more intensive reaction than that of an elderly subject) and the condition of the skin should be taken into account.

If the control test with the extracting fluid should not be completely negative, there will have to be a marked difference if any value is to be attached to the specific reaction.

The Prausnitz-Küstner test may be performed as a control in doubtful cases (cf. Chapter V). This test will have to be resorted to occasionally in the case of patients with disturbances of the autonomic nervous system. The skin is so sensitive in some of these cases, that practically all reactions are positive.

Skin reactions should always be read where the lighting is best in the surgery, the light falling obliquely on the reaction; moreover, it is advisable to take the readings at the same time of day in the same patient.

Therefore, suitable artificial light should preferably be used in reading reactions.

Personal investigations

In order to determine the maximum *quantity of liquid* that may be injected intradermally, injections of physiological saline solution followed by injections of Coca's solution (R/NaCl 5.—, $NaHCO_3$ 2.5, phenol 4.—, distilled water to 1.000) were made in 20 volunteers, 0.01 ml., 0.02 ml., 0.03 ml., 0.04 ml., and 0.05 ml. respectively being injected in each case. Within 20 minutes after injection, an examination was made to determine the largest quantity that may be injected without causing redness. The maximum quantity was found to be about 0.02 ml. for either solution. Larger doses caused marked (non-specific) irritation in some cases.

Conclusion: 0.02 *ml. is advisable in intradermal injections.*

With Hansen [4] and Kräpelien [33], the injection of *histamine* is believed to be a useful standard in classifying skin reactions. In that case, however, a fixed amount, viz. 0.02 ml. of 1 : 10.000 histamine, should be injected. The average size of the resulting wheal is 1.8 by 1.2 cm., the average size of the areola being 4 cm. by 3.5 cm. The histamine test usually is negative in dilutions below 1 : 1000.000. *Therefore*, 1 : 10.000 *histamine is the most suitable standard for* +++, 1 : 100.000 *for* ++ *and* 1 : 1000.000 *for* +.

As a rule, *pseudopods* at the site of the wheals are regarded as an intensive reaction. Some add one or several plus signs in recording the intensity of the reactions in these cases.

The present author happened to have the opportunity of making a comparison in classifying these pseudopods.

The patients in question were two patients with pollinosis. Each showed a very intensive reaction and pseudopods following the intracutaneous injection of 0.02 ml. of 100 units of grass pollen extract (1 gram of dry grass pollen contains 1.000.000 pollen units = Noon units). If there had been no pseudopods, the reaction would have been classified as +++. The two patients developed a mild shock following the injection, one responding with mild urticaria and the other with an attack of hay fever. The serum of these two patients was then used to perform a Prausnitz-Küstner test, the results being compared with those obtained in testing with the serum of a patient with hay fever in whom the skin reaction was +++, pseudopods being absent. A 1 : 100 dilution of the serum was found to be necessary to produce a similar degree of redness in the passive transfer test. Therefore, two plus signs were added in classifying reactions in which pseudopods were present.

Conclusion: When pseudopods appear at the site of a wheal produced by an injection, ++ should be added in classifying the skin reaction.

Inquiry

To ensure that the data available in testing for allergens should be as reliable as possible, an international inquiry was instituted, in which 100 colleagues were requested to classify the skin reactions as shown in the accompanying figure. Of the questionnaires, 30 were filled out and returned. The results have been listed on Table II. The classification of the reactions varies widely; the smallest: from negative to ++; the next reaction: from negative to ++++; the third: from ± to ++++ ++ (6 times +) and the largest: from + to +++++++++ (8 times +). Several investigators measure the transverse section of the wheal and erythema, but this is also done by various methods. The standards adopted by Kräpelien [33] in the classification of skin reactions in adults are different from those adopted in children. In classifying skin reactions in the latter, an injection of 0.05 ml. of a 1 : 10.000 solution of histamine is designated as +++; the reactions are classified in comparison with this histaminic reaction.

Salén and Juhlin Dannfelt [33] only measure the *wheal* and not the areola surrounding it. They take the largest diameter and the largest diameter perpendicular to it. The sum of these diameters is noted. When the wheal has increased in size, but is not more than 10 + 10 mm. or 20 mm. together, the reaction is designated as ±, when the sum is up to 30 mm., it is designated as +, up to 40 mm. as ++, up to 50 mm. as +++, etc.; this is *only* so when 0.1 ml. of extract has been injected intracutaneously.

Brown [33] uses one plus sign to designate the smooth round wheal which is the standard of the skin test and which does not change. When the wheal increases in size, but continues to be round without pseudopods, the reaction is classified as ++; as soon as pseudopods appear, the reaction is classified as +++. When the wheal increases to twice its size at this point, the plus signs are also doubled. Brown suggests classifying the second reaction as 3 to 4 +, as he is not sure whether pseudopods have appeared with the first reaction. As a result, the third reaction would become 6 + and the fourth at least 8 +. The largest reaction is classified as 10 +.

Brown assumes the smallest reaction following an intracutaneous injection to be characterized at least by a round wheal surrounded with an areola, as these will always result from the injection of the smallest quantity of extract.

Freeman [33] states that the reactions cannot be designated as +, ± or —. Freeman measures the surface area of the wheal in sq.mm. Assuming the innermost ring to be the outline of the wheal, then the size is measured as follows:

Dear Colleague,

Will you kindly state your classification of these intracutaneous reactions? Why did you classify them as: —, ±, +, ++ or +++?

Thanking you sincerely,

(van der Bijl)

TABLE II

Results of Inquiry

1	+	++	+++	++++	Bencard testing chart.
2	—	±	+	++	
3	—	—	±	++	mm +
4	—	±	+	++	
5					
6	0	14	58	123	sq. mm
7	—	—	+	++	
8	—	+	++	+++	
9	—	+	+±	++±	
10	—	+	+++	+++	
11					metric system
12	—	±	+±	++±	
13	—	±	2/5	3/6	
			++	+++	
	wheal up	to 2 mm	up to 3 mm	> 3 mm	in comparison with histamine
14	—	±	+	++	in comparison with histamine
15	±	+	++	+++	
16	—	—	+	+++	
17	—	+±	+++	++++++	
18	—	±	++	+++	area of redness (not size of the wheal)
19	—	±	+	+	
20	—	±	+	+±	
			14 + 6	13 + 13	
21	—	+	++	+++	
22	±	+	+++	+++	
			(pseudop.)		
23	—	+	++	+++	pseudop. = another +
		(10 mm)	(15 mm)	(720 mm)	
24	±	+	++	+++	
25	±	+	++	++++	
26	—	+	++	+++	combined erythema and wheal
27	++	++++	++++++	8 × +	
28	—	±	+	+	
29	—	+	+++	++	
30	±	+	++	+++	(B. Bloch)

Reaction 1 = nil, reaction 2 = 14 sq.mm., reaction 3 = 58 sq.mm. and reaction 4 = 123 sq.mm. In view of the more recent data on allergic disease, Freeman believes that any clinical interpretation of these reactions will depend on a large number of factors and cannot be classified as + or —.

Orie [33] and Ten Cate [17] base their classification of the size of the wheal on the 'mean diameter' which is the sum of half of the largest diameter extending across the needle puncture and the middle perpendicular to the largest diameter.

This is 5 mm. in a wheal produced by the injection of 0.025 ml. of extract.

Orie and Ten Cate classify reactions as doubtful (±) when there is only erythema.

When the wheal is from 5 to 7.5 mm. in diameter = +
" " " " " 7.5 to 10 mm. in " = +±
" " " " " 10 to 12.5 mm. " " = ++
" " " " " 12.5 to 15 mm. " " = ++±
" " " " " over to 15 mm. " " = +++

Kämmerer [6] represents the results of the tests in graphs in which the size both of the wheal and of the erythema are accurately recorded in mm., the former by lines in bold type, the latter by a thin line.

Berkoff [3] has suggested the use of a standard scale called the 'cutometer'.

Round holes are made in a piece of celluloid or X-ray film about 10 by 16 cm.; in the left half, holes of 1, 1.5, 2 and 3 cm. in diameter, the holes made in the other half measuring 0.5, 0.75, 1.25, 1.75 and 2.5 cm. in diameter. The holes in the left half are used for measuring the sizes of the wheal in the reactions classified as 1+, 2+, 3+ and 4+, those in the other half being used to measure the sizes in the ± reactions.

The erythema can be read by drawing a network of horizontal and vertical rulings on the film at intervals of 1 cm.

Wodehouse [3] suggested expressing the results of the skin reactions in units, so-called cutaneous reaction units, *C.R.U.*

The C.R.U. is the size of the erythema in mm. minus the size of the wheal, divided by 10, for instance:

$$(30 - 15) \times 15 : 10 = \frac{15 \times 15}{10} = \frac{225}{10} = 22.5; \text{ C.R.U.} = 22.5$$

The quantity of allergenic extract injected is a very important factor which has to be taken into account in considering the differences in the classification of skin reactions.

Ten Cate [17] injects about 0.025 ml., so that a wheal measuring 5 mm. in diameter results; Niemand-Andersen [36] suggests injecting 0.1—0.2 ml. and compares the reaction with that following the injection of 1 : 1.000

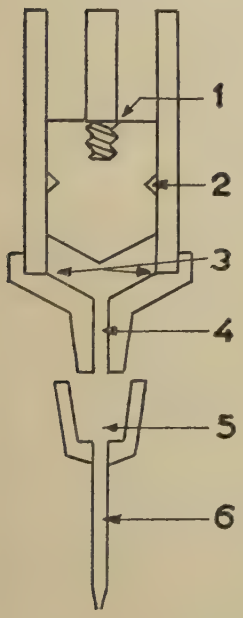
histamine. Hansel [3] recommends injecting a quantity producing a wheal about 3 to 4 mm. in diameter, instead of injecting 0.01 to 0.02 ml., Vaughan and Black [11] also suggest 0.01 to 0.02 ml.; Cooke [1] suggests 0.02 ml.; Hansen [4] recommends 0.02 to 0.05 ml. intradermally and Kämmerer [6] writes: 'the amount of liquid injected should not exceed 0.1 ml.'

In view of the differences appearing in the inquiry and those reported in the literature, the personal method of classifying the size of the wheal is preferred, as it is based on empirical data. It would be advisable generally to adopt this standard to enable a comparison of results on an international scale.

CHAPTER III

FREEING SYRINGES AND NEEDLES FROM ALLERGENS

The practitioner is constantly faced with the question of how to free his syringes and needles from allergens. Bacteriological sterilization and the keeping sterile of needles and syringes is essential to testing. The removal of allergens is a problem to which little attention has been paid so far, although it is a matter of vital importance. When record or tuberculin syringes are employed, allergenic extracts may accumulate in the following places:



1) at the head of the plunger, at the point where it is attached to the handle (often by screwing);
2) in the middle of the plunger, in the grooved portion;
3) at the junction of glass and metal in the syringe;
4) in the cone of the syringe;
5) in the cone of the needle;
6) in the needle itself.

There are two questions of considerable practical importance:

1) Will it be sufficient to cleanse used syringes with a phenol or Coca's solution and may they then be used again to inject another allergenic extract?
2) Can a syringe not only be sterilized, but also freed from allergens, by mere boiling?

Personal investigations

In 4 patients with pollen allergy and 7 who were hypersensitive to house dust, the following experiments were carried out:

1) The first injection of an allergen was made as usual, 0.02 ml. of a 1 per cent extract of house dust or 0.02 ml. of 1.000 units of pollen extract being injected.

2) The syringe, which had previously been filled with the allergenic extract, then was filled ten times with Coca's solution, which was squirted out again. In conclusion, 0.25 ml. of Coca's solution was drawn into the

syringe and the plunger was moved up and down ten times as far as the 1 ml. mark, so that the contents was in close contact with the syringe. This solution was used to perfo rm an intracutaneous test.

3) The allergenic extract was drawn into the 1 ml. tuberculin syringe and immediately ejected again. The syringe then was boiled at 100° for thirty minutes and finally filled up to one fourth with Coca's solution. The plunger was moved up and down ten times and the resulting mixture was injected in the patient.

4) The allergenic extract was drawn into the 1 ml. tuberculin syringe and immediately ejected again. The syringe then was boiled at 100° for thirty minutes and finally filled up to one fourth with Coca's solution. The plunger now was no longer moved up and down, the mixture obtained following 5 minutes' contact with the wall being injected in the patient.

Once again, only those cases have been included in which the control injection was found to be negative.

The following results were obtained:

TABLE III

		1	2	3	4
Grass pollen, 1.000 units	1.	+++	±	—	—
	2.	++++	+	—	—
	3.	+++	+±	+	—
	4.	++	+±	+	—
Total:		12+	4½+	2+	0
		1	2	3	4
House dust 1 per cent	1.	+++	+±	+±	±
	2.	++±	++	+±	+
	3.	++++	+	+±	—
	4.	++±	+±	±	—
	5.	++++	+±	±	—
	6.	+++	+±	±	±
	7.	++++	++	+	±
Total:		23+	11+	7+	2½+
Total house dust + pollen:		35+	15½+	9+	2½+

The above experiments clearly show that it is very difficult to free a syringe from allergens. For when the syringe is not boiled, fairly intensive skin reactions are found to occur. Even when it is boiled for thirty minutes, a considerable amount of allergenic extract is left behind in the syringe.

When testing regularly for allergens, it therefore is advisable to number and label the syringes, needles and vials of allergenic extract, the same

syringe and needle being used for each allergenic extract. If this is not done, another method of cleaning the syringes and needles should be devised.

Efforts were made to develop a simple, but nonetheless fairly reliable method of freeing the material from allergens by:

a) attempting to avoid errors in boiling;
b) examining whether the syringes might be freed from allergens by chemicals;
c) studying the effect of the pH of the rinsing fluid;
d) using higher, if need be very high temperatures.

a) In boiling the syringes, it is a mistake to place them horizontally in the pan. In that case, will the boiling liquid circulate sufficiently through the interior of the syringe? And what happens if an air bubble has remained in the syringe when it is in an approximately horizontal position? In several cases, these air bubbles are not even displaced during boiling. Mossel [32] states that heat penetration is inadequate in air pockets. The boiling fluid should constantly flow through both the needles and the syringes (under pressure). Accordingly, they should be treated in the vertical position. This method will, however, only be possible economically in large hospitals. (A so-called 'pressure cooker' was never used in testing by the present author, as reports in the literature do not show whether this method will produce sufficiently reliable sterilization.)

b) Chemical cleaning should always be accompanied by mechanical cleaning. To begin with, rough cleaning of the syringes and needles will be essential. This should never be done by water, as in that case the blood residues will be precipitated. The syringes should be cleansed with 3 per cent H_2O_2 to decompose the blood residues, if any, and then with a physiological saline solution or 0.5 per cent phenol. This is followed by *mechanical* cleaning, brushing of the syringes with a surface-active substance such as typol (a sulphonal anion), lodaline or desogen. Various chemicals may serve as disinfectants. Lyes cannot be used, as the metal of the syringes is affected by these agents. Soda also has injurious effects in the long run.

The following methods may be used:

1) Boil the syringes and needles for forty-five minutes, then pour off the water, rinse the instruments and boil again for forty-five minutes (acetic acid having been added), pour off the water again and finally wash in pyrogen-free water.

The syringes are then wrapped in cellophane, heated at 110^0 in the steam sterilizer and dried again in the autoclave.

2) Coca and Milford suggest boiling the instruments for 15 minutes in 2 per cent washing soda (sodium carbonate) after mechanical clean-

ing, then cleaning them with lukewarm water and brushing them again, followed by boiling in a hydrochloric acid solution (21 ml. of distilled water to 50 ml. of a 1 per cent solution of concentrated hydrochloric acid). The syringes are then washed and rinsed twice in distilled water; this is followed by sterilization in the autoclave.

There are several other methods of disinfection, e.g. using gemton, lissapol and mixtures of lissapol and other chemicals such as FLM 50: a mixture of lissapol (ICI) with phosphates. All these methods take a fair amount of time and are cumbersome. Practical experience in testing for allergens has shown, however, that rough cleaning is an essential procedure.

Personal investigations

In order to study the results that may be obtained in the various methods of cleaning, the following injections were made experimentally in a number of subjects: (All syringes had been filled with the same allergenic extract for thirty minutes)

1) injection of the allergenic extract;
2) injection of Coca's solution in a syringe brushed with 5 per cent desogen;
3) injection of Coca's solution in a syringe brushed with lodaline and then boiled for thirty minutes in a lodaline solution;
4) injection of Coca's solution in a syringe brushed with 5 per cent desogen and then boiled for thirty minutes in water;
5) injection of Coca's solution in a syringe brushed with lodaline and then boiled for thirty minutes in water;
6) injection of Coca's solution in a syringe brushed with distilled water and then boiled for thirty minutes in water;
7) injection of Coca's solution in a syringe cleansed ten times with 70 per cent alcohol plus 5 per cent formaldehyde;
8) injection of Coca's solution in a syringe cleansed ten times with 5 per cent desogen and 1 per cent sodium nitrite;
9) injection of Coca's solution in a syringe cleansed with Galli-Valerio's solution: R/35 per cent formaldehyde solution 25, phenol liquefactum 5, sodium borate 15, distilled water to 1.000 ml.;
10) a control test.

Conclusion: These experiments show that syringes mechanically cleaned and then boiled (Nos. 2 to 6 inclusive) *produce hardly any positive reactions in skin testing, in contrast with the instruments that were only cleansed* (Nos. 7 to 9 inclusive). *It probably makes little difference which disinfectant is used in mechanical cleaning* [Table IV].

c) Does the pH of the rinsing fluid play any part in freeing the syringes from allergens?

Criep, Scheparte and Engel [18] found that the pH of allergenic extracts stored for 6 months in the refrigerator *had* altered, though the allergenic action had not diminished.

TABLE IV

Case	1	2	3	4	5	6	7	8	9	10
1	+++	—	—	±	—	—	+	+	—	—
2	++±	—	—	—	—	—	—	±	+	—
3	++	—	—	—	—	—	±	—	—	—
4	++±	—	—	—	—	—	—	+	+	—
5	+±	—	—	—	—	—	—	—	—	—
6	++++	—	±	±	±	±	±	—	±	—
7	+++	±	—	—	—	—	—	+	±	—
8	++±	—	—	—	—	—	—	—	+	—
9	+++	—	+	—	—	+	±	—	+	—
10	++	—	±	—	—	—	±	—	—	—
11	++	—	—	—	—	—	—	±	—	—
12	++	—	—	—	—	—	—	—	+	—
13	++	—	—	—	—	—	—	—	—	—
14	++±	—	—	—	—	—	+	+	—	—
15	+±	—	—	—	—	—	—	—	—	—
16	++±	—	—	—	—	—	—	—	+	—
17	+++	—	±	—	—	—	+	—	±	—
18	++	—	—	—	—	—	—	—	—	—
19	++	—	—	—	—	—	—	—	—	—
		½	2½	1	½	1½	5	5	7½	0

Increasing the temperature also had little effect on the pH and changes in the pH of a ragweed extract hardly altered the skin reactions in allergic and non-allergic patients.

Though heating of *glass* results in alkalisation (Esty and Catheart [12]), this does not affect the results obtained in skin tests.

The role of the pH is best studied by preparing allergenic extracts having a particular pH and using them in skin tests (cf. Chapter VI).

d) The degree of restistance of allergenic extracts to high and very high temperatures is discussed in greater detail in Chapter VI.

Summary: The following treatment of instruments contaminated by allergenic extracts is essential:

To begin with, a mandrin should be passed through the needles, both the syringes and the needles then being cleansed with 3 per cent H_2O_2; this is repeated with 0.5 per cent phenol. This is followed by mechanical cleaning, i.e., brushing with typol or desogen, and the instruments are rinsed again with 0.5 per cent phenol. The syringes and needles then are treated with preliminary boiling in a pan for *five minutes*, the needles being suspended in a gauze net. The instruments are removed from the pan after these five minutes and allowed to drain, and then are boiled again in another pan for thirty minutes.

Even when cleaning has been highly effective, it will be advisable to keep the syringes and needles to be used in skin testing apart from the syringes used in injecting adrenaline and in desensitization, as therapeutic extracts frequently are stronger than diagnostic extracts.

Syringes and needles free from allergens may be stored free from bacteria in sterile lidded receptacles filled with:

a) 70 per cent alcohol plus 5 per cent formaldehyde (Thorer),
b) the Galli-Valerio solution,
c) a 5 per cent solution of desogen (Geigy) to which 1 per cent sodium nitrite may be added (Baumann [14], Grumbach),
d) zephirol (Bayer).

c) and *d)* bear some resemblance to one another.

Personal investigations

It is of importance to know what may happen when a 5 per cent desogen solution and zephirol are handled carelessly ,as the two liquids are for external use only. Therefore, 0.02 ml. of various dilutions of a desogen solution were injected intradermally in a guinea pig. A 1 : 1.000 dilution did not produce any changes, a 1 : 500 dilution causing doubtful redness, a 1 : 200 dilution causing marked redness, a 1 : 100 dilution causing very marked redness (++) and a 1 : 10 dilution causing +++ redness and central necrosis.

Blood of the present author underwent haemolysis following the addition of 1 : 800 desogen.

Remnants of the blood of two patients were mixed with desogen in various concentrations. A 1 : 800 dilution was found to cause haemolysis, whereas a 1 : 1.000 dilution did not do so.

Prausnitz-Küstner tests performed with:

a) 100 units of grass pollen plus 0.5 per cent phenol plus 1 : 1.000 desogen,
b) 100 units of grass pollen plus 1 : 1.000 desogen,
c) 100 units of grass pollen plus 0,5 per cent phenol,

did *not* show any decrease of the allergenic action in the present author.

Intracutaneous injection of 0.02 ml. of 1 : 1.000 desogen did not pro-

duce any non-specific irritation in a volunteer or the present author within thirty minutes. Accordingly, a 1 : 1.000 dilution, and not the 5 per cent solution, is the limit.

Now desogen is not a specific anion or cation, so that either the allergen is not precipitated by the desogen or it rapidly dissolves again on precipitation.

Accordingly, desogen alone will not free a syringe from allergens, though it may be used as a preserving fluid.

Conclusion: A syringe taken from a preserving fluid should first be cleansed to avoid non-specific irritation in intracutaneous testing. Coca's solution is used for this purpose and the syringe is allowed to drain.

The plunger should then be pushed up and down a few times, the syringe being directed downwards, before attaching the needle. Prior to testing, the needle should be blown through a few times rather than cleared by sucking.

CHAPTER IV

REACTIVITY OF THE SKIN

It will partly depend on the degree of reactivity of the skin whether or not skin tests are positive. Disregarding the factors that may affect this reactivity is one of the causes of the variability of skin tests.

The most marked reactions are observed on the back. Nevertheless, the arm or, if need be, the thigh are to be preferred in routine testing (using the thigh, however, slightly increased the risk of infection). The arm as well as the thigh have the great advantage that the extremity may be ligated in an emergency, so that systemic reactions may be prevented.

Vaughan [11] states that both *local anaesthesia* and *chilling* have an adverse effect on the reactivity of the skin.

The method used in disinfection of the skin is also believed to be an important factor.

A) DISINFECTION OF THE SKIN

Vaughan writes: 'Simple cleansing of the skin with soap and water, if necessary, is sufficient. No antiseptic need be used. After twenty years the writer has not yet seen an infected test site, following either scratch or intracutaneous testing.'

The present author cannot agree with this statement.

Any action in which the normal continuity of the skin is ruptured and in which substances are introduced into the living tissues of the skin (which occurs in both intracutaneous and scratch testing) involves the risk of infection. Accordingly, measures should be taken to prevent the entry of pathogenic organisms.

To begin with, the sebum should be removed in disinfecting the skin, as it contains the largest number of bacteria. This should undoubtedly be followed by disinfection, the more so as pricking or abrading may occasionally be too deep despite a correct technique.

The usual and most reliable method of disinfection by iodine is unsuited for this purpose, as the colour of the iodine prevents reading of the erythema. The following disinfectants are recommended by Cooke [1], Feinberg [2] and Hansel [3]: alcohol, ether, cetavlon, zephiran, etc.

The fact should be stressed, however, that neither ether nor alcohol are adequate disinfectants.

Ether is more effective than alcohol in removing the grease from the skin, resulting in the disappearance of the superficial flora. The deeper-seated (resident) flora of the skin contains few organisms likely to be

pathogenic for the individual in question. It might be, however, that ether, rather than alcohol, adversely affects the result of the test, i.e. that the local anaesthesia following the chilling might be unduly prolonged.

Winkler [33] states that ether evaporates completely within two minutes, so that there will no longer be any anaesthetic effect. When the quantity of ether used is not too large, the hyperaemia will also disappear rapidly.

Personal investigations (2 diagrams)

1) The skin reactions in a number of patients following washing with soap and water, and cleansing with spirit, 96 per cent alcohol and ether were compared [see Table V].

TABLE V

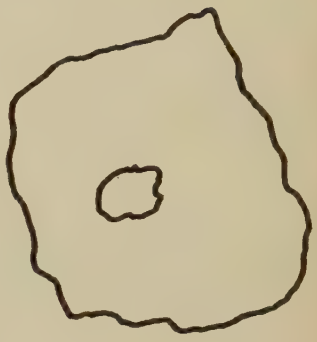
Comparison of skin reactions read within 20 minutes after the intracutaneous injection of 100 U. of grass pollen extract. The skin was cleansed with soap and water, spirit, 96 per cent alcohol and ether prior to testing.

Case	soap and water	spirit	96 per cent alcohol	ether
1.	++	++	++	+±
2.	+	++	+±	++
3.	++	+±	++	++
4.	++	++	++	++
5.	++	+	+±	++
6.	—	++	+	+
7.	—	+	+	+±
8.	++	+	+	+
9.	+	++	++	+
10.	+±	++	+±	+
11.	+±	+	+	++
12.	++	+±	+	+±
	17	19	17½	18½

Conclusion: There is no difference in the size of the reactions following cleansing of the skin with agents producing chilling and anaesthesia.

Therefore, there is no objection to proper cleansing of the skin with ether and alcohol. To ensure disinfection, the subsequent use of zephiran desogen etc. may be recommended.

I
The liquid was injected into the dry, uncleansed skin



II
The skin was cleansed with:
Ether

III
The skin was cleansed with:
Spirit

IV
The skin was cleansed with:
Alcohol (96 per cent)

0.02 ml. of a 1 per cent extract of fungi injected immediately after cleansing of the skin

I
The skin was cleansed with:
Spirit

II
The skin was cleansed with:
Alcohol (96 per cent)

III
The skin was cleansed with:
Soap and water

IV
The skin was cleansed with:
Ether

Control test

0.02 ml. of a 1 per cent extract of fungi injected 2 minutes after cleansing of the skin

2) There also was no difference in the size of the skin reactions when the skin was treated with spirit, 96 per cent alcohol and ether prior to testing, all liquids being applied locally for two minutes.

3) To determine the effect of prolonged chilling and heating of the skin on the reactions to allergens, 0.02 ml. of 100 units of grass pollen were injected at three sites placed 6 cm. apart on the forearm in five patients with pollinosis. The upper test site served as a control, the middle site had been chilled with an ice-cube and the lower site had been heated to 40^0. Heating and chilling were discontinued after twenty minutes and the skin reactions were studied for some time.

Initially, the size of the erythema and the wheal, was found to differ markedly from the normal size, both being smallest on the chilled skin and largest on the heated skin. All variations of the erythema, however, disappeared within from 13 to 24 minutes. The wheal continued to be enlarged for a prolonged period (up to about thirty minutes) on the heated skin.

4) The skin reactions in these patients were also studied following chilling or heating of the skin for twenty minutes prior to making these injections.

There no longer was any marked difference of redness within twenty minutes after injection, though the wheal on the heated skin was slightly larger.

Although conditions of nature were not reproduced in these experiments, it may be concluded that oud-patients should be allowed to remain in an adequately heated waiting-room for thirty minutes prior to skin testing in winter.

B) THE EFFECT OF DRUGS ON SKIN REACTIONS

Another question frequently asked is: what is *the effect of drugs*, especially antihistaminics and adrenaline, on the size of the skin reactions?

Anderson does not consider skin testing advisable when patients have used ephedrine or aminophylline in the form of suppositories or antihistaminics within the past 8 hours or when they have been given adrenaline or isopropylnoradrenaline sublingually, aminophylline or euphylline intravenously or when they have been treated with other sympathomimetics within the last 4 hours. Calcium compounds, chlorides and sodium chloride, atropine, ACTH and cortisone are not believed to have any effect on skin reactions.

Others report the effects of 50 mgr. of ephedrine sulphate given orally, of 0.65 mgr. of atropine sulphate administered subcutaneously and of 0.86 mgr. given orally as being doubtful. Intravenous administration of 10 ml. of calcium gluconate and 10 ml. of sodium iodide in the usual concentrations did not have any inhibitory effect. This was verified by Tuft and Brodsky [42]. These authors state that the effects of ephedrine,

asthmolysin, pituitrin and euphylline are doubtful. The period for which skin reactions become smaller when posterior lobe extracts have been injected subcutaneously is assumed to be about sixty minutes.

Cooke [1] reports epinephrine given within sixty minutes as resulting in smaller or negative skin reactions, inhalation of this drug having no effect on skin reactions.

Smyth and Bain [37] also found that skin tests may become negative owing to the administration of epinephrine; this was verified by Mosko and Marshall.

The present author did not observe any changes in the skin reactions following administration of ephedrine hydrochloride (25 mgr. given orally), aminophylline (240 mgr. given orally), 10 ml. of 10 per cent calcium gluconate and 10 ml. of calcium gluconolactobionate, both given intravenously.

Ten Cate [17] also believes that antihistaminics have an inhibitory effect.

Feinberg [2] recommends administering small doses of these drugs in cases of marked dermographism. This is assumed to reduce general reactivity, so that specific reactions will become more marked.

Lecomte [28] observed an inhibitory effect of local anaesthetics and antihistaminics on the response to tuberculin when they were injected *intradermally at the same time* as the tuberculin. Mosko and Marshall found that benadryl and pyribenzamine did not have any effect on the skin reaction if the test was done within 15 minutes after injection of the drug. If given within 30 minutes, however, an inhibitory effect was observed.

Personal investigations

a) Thephorin

In order to be able to form an opinion on this matter, an intracutaneous test was performed in a number of patients who were definitely sensitive to certain allergens. The reaction was read and traced after 20 minutes. The patient was then given 50 mgr. of thephorin orally. Sixty minutes later, a similar intracutaneous injection was made on the other arm at the same level, the result being traced again and compared after 20 minutes [TABLE VI].

The skin reactions did not show any significant changes in six cases and had markedly increased in size in four cases. The *initial* reaction also was more marked in some cases, no side-effects being observed.

Accordingly, a number of authors assume that administration of certain antihistaminics has only a slide inhibitory effect. Personal studies did not show this to be the true of thephorin; on the contrary, this drug increased the reaction in some cases.

b) Adrenaline

Another question is that of the type of skin reaction following the injec-

TABLE VI

Case	Allerg. extr.	time of 1st inj. 8:40 a.m.	read at 9:00 a.m.	theph.50 mgr. at 9:00 a,m.	2nd inj. at 10:10 a.m.	2nd inj. read at 10:30 a.m.	1st inj. read at 10:30 a.m.
1	grass pollen 100 U rye pollen 100 U maize pollen 100 U		+++ +++ +++			+++ +++ +++	
2	house allergen 1 %		++			+++	
3	house allergen 1 %		++			++	
4	rye pollen 100 U seed allergens 1 % grass pollen 100 U		++++ +++ ++			+++ ++ +++	
5	fungi 1 % sauerkraut 1 % rye pollen 100 U histamine 1 : 100,000		+++ +++ ++ +±			++ +± + ±	+ + +++ ++++
6	house allergen 1 % fungi 1 % rye pollen 100 U		++++ ++ +++			+++ +++ ++±	++ ++ +±
7	house allergen 1 % May 6 house allergen 1 % May 7 house allergen 1 % May 8 rye pollen 100 U seed allergens 1 % histamine 1 : 100,000		++± ++ ++ ++ ++ +			++± ++± +± + + +	+++± ++ + +
8	fungi 1 %		+±			+	+
	total		53½+			46+	23+ [1]

[1]) 23+ in 12 cases = 43+ converted.

tion of 0.5 ml. of 1 : 1.000 adrenaline. Once again, skin tests were performed in 13 patients known to respond to a particular allergenic extract. The reactions were read and recorded after 20 minutes. This was immediately followed by subcutaneous administration of 0.5 ml. of 1 : 1.000 adrenaline, another skin test of the same type being made at

the same level on the other forearm 30 minutes later and the reaction being read and recorded under constant control after 20 minutes.

Almost all patients showed a distinctly smaller skin reaction following the injection of adrenaline. The intracutaneous injection of the allergen made *prior to* the injection of adrenaline also produced reactions which became smaller within a shorter time than usual in the greater part of the cases.

In order to gain a better insight into this problem, the tests were performed again in 7 patients, the second test being done within 60 rather than 30 minutes after the injection of adrenaline. The skin reactions were *not* found to have become smaller in these cases.

Conclusion: Injection of adrenaline markedly reduces the size of the skin reaction. The affect of an injection of adrenaline, however, does not persists beyond 60 minutes.

A number of the factors which may affect skin reactions and which were reviewed on p. 4, will be discussed in greater detail.

As early as 1929, Storm van Leeuwen and Van Niekerk [40] stressed the fact that testing to allergens should be done during the period when the patient is free from attacks. The patient is emotionally upset during attacks. This results in contraction of the smooth muscle fibres and vascular changes may occur as well.

These factors may give rise to severe non-specific reactions in testing, which reactions actually are in no way associated with the allergic condition. In interpreting skin reactions, the nature of the symptoms should therefore be taken into account; they may be intermittent or they may be chronic and characterized by exacerbations. Even then, however, the reactivity of the skin shows marked differences.

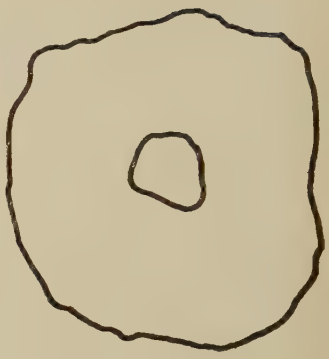
C) DAILY VARIATIONS IN THE REACTIVITY OF THE SKIN

The question arises: what are the reactions shown by patients *on different days?*

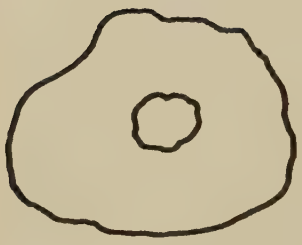
Personal investigations

1) In order to study this matter, a particular allergenic extract was injected subcutaneously on the volar surface of the forearm during a number of days in 4 hospitalized patients under conditions that were identical wherever possible (i.e., in the same room, at a constant temperature, at the same time, by the same assistant using the same syringe and needle and injecting in the same direction by employing the apparatus as described in Chapter V (p. 46), compression being exerted only during injection). *In these cases, the skin reactions were found to differ markedly on consecutive days.* These daily variations, however, might be due in part to the fact that the tests were not performed at the same site, but

Feb. 4, 1957



Feb. 5, 1957



Feb. 6, 1957

Feb. 7, 1957

Feb. 8, 1957

0,02 ml. of a 5 per cent extract of house fungi was injected on various days

about 6 cm. apart, or to technical errors such as the making of more or less deep intracutaneous injections. For a series of these tests, see page facing.

D) SEASONAL VARIATIONS IN THE REACTIVITY OF THE SKIN

There are not only daily variations, however, for, as early as 1947, Stier [39] pointed out that the skin reactions will depend on the *season* and Hansel [3] advanced the theory that a patient will also become more sensitive to other allergens during the season in which his 'principal allergen' gives rise to the onset of allergic manifestations.

Personal investigations

1) In order to study this matter in greater detail, a complete series of tests were performed in 91 patients, both in the period during which they showed the largest number of symptoms, i.e. *during their season*, and outside this period, i.e. about 5, 6 or 7 months later. In the interval, the patients only received symptomatic treatment and were not desensitized, little attention being paid to elimination diets outside the season. Seventy-three food allergens and thirty-four inhalants were tested in these cases, the average number of positive tests being 10 and 7 respectively. The ratio between the incidence of positive tests in the season of the patients and that at other times of the year was found to be 3 : 2 in the case of food allergens and 7 : 4 in the case of inhalants.

Accordingly, the season apparently has a slightly more marked effect on inhalants than it has on food allergens.

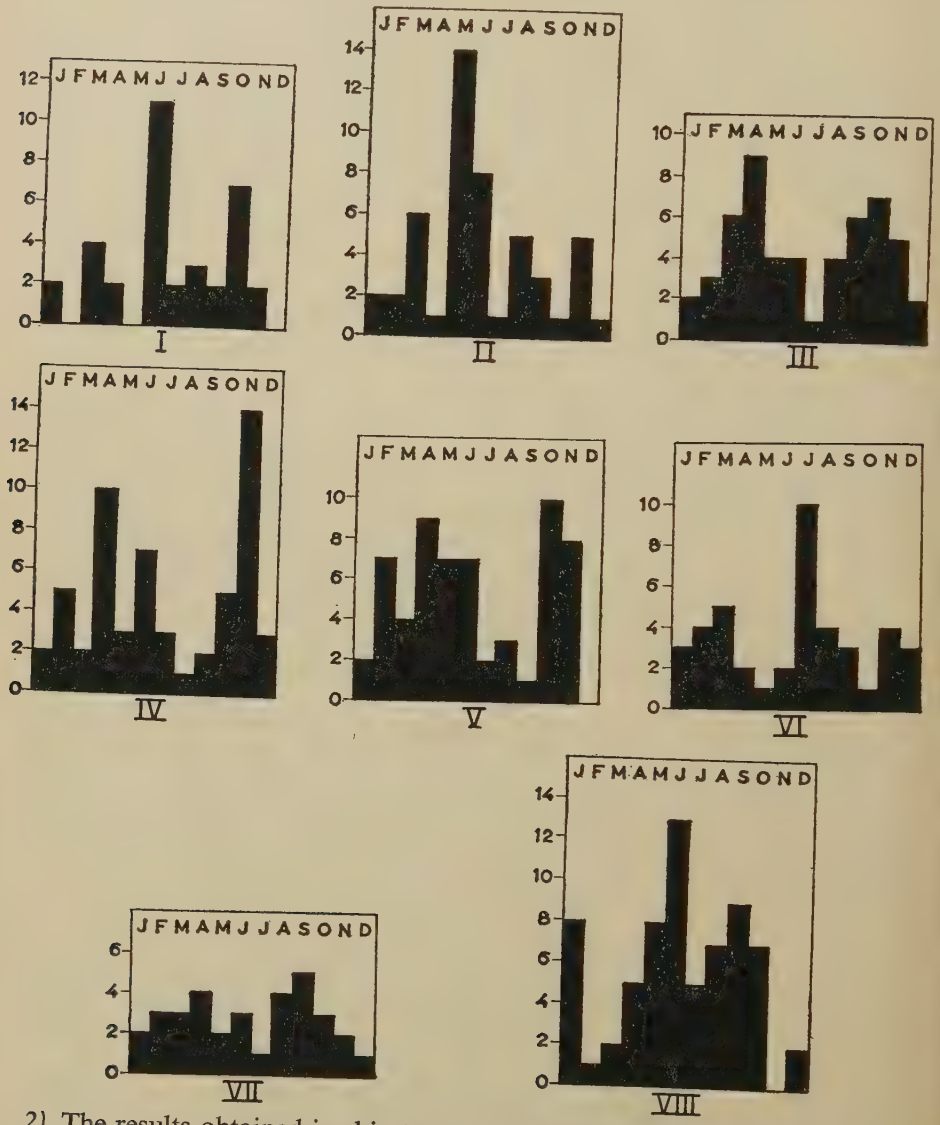
The patients were selected to some extent in this experiment to ensure a maximum degree of reliability in testing. Of the patients, 39 were women; 26 were from the country, 26 from small towns and 39 lived in Amsterdam. The number of men in the Amsterdam group was twice as large as that of the women, as the former usually are more liable to allergenic variations than are women. All patients were over 16 and under 60.

Of the two complete test series performed in these 91 patients, the incidence of positive skin tests, both to foods and inhalants, as compared with the monthly average during the course of the year, was highest in the *autumn* and lowest during the period from February to April inclusive. The 'peak' incidence of positive reactions to food allergens occurs a month before the inhalant peak. The fact that the latter occurs some time later probably is due to fungi. Apparently, a large number of subjects are sensitive to certain allergens during a particular period; in the Netherlands, for instance, to fungi in September.

It would be interesting to see whether routine testing will reveal other 'variations' as well.

TABLE VII

Survey of the incidence of monthly variations of individual allergenic extracts in comparison with the group extract during 1956.



2) The results obtained in skin tests personally performed in 275 *recent* cases *during the course of a year* were accurately recorded and the month in which testing took place was noted.

The results obtained with group extracts (cf. pp. 79—81) were compared with those obtained using solutions of individual allergens of this

group, both in the case of inhalants and of food allergens. There were 8 sittings per patient, which took place at intervals of from 2 to 3 days.

Examination of these monthly data during the course of the year shows that variations occur, which may be due, among other things, to very intensive skin reactions in patients with seasonal allergy. They may also be due to the fact that the allergenic extract causes non-specific irritation [Table VII].

A comparison of the curves shows a peak in group I in June; this probably was due to the fact that the out-patients did not only consist of new patients with hay fever, but also of workers from the bulb district showing typical bulb dermatitis. Group II is characterized by a peak in May, which may be due to spring-cleaning in the Netherlands. Group III shows a peak in April as well as in October. The October peak may be attributable to fungus allergy, the April peak to farm inhalants. The food allergen curves are highly irregular. The peaks in this case cannot be accounted for.

3) In order to enable a more accurate evaluation of seasonal factors, all positive and negative skin tests in 300 patients were recorded every month in 1957 and 1958. The incidence of cases during the various months varies considerably. In 1957, the number of patients from January to December inclusive was: 57, 39, 17, 14, 13, 13, 13, 22, 24, 27, 22 and 39 respectively, and in 1958: 34, 33, 22, 14, 11, 19, 20, 17, 31, 26, 36 and 37 respectively.

To enable a comparison, the so-called conversion number, into which every number of cases in each month was divisible, was calculated for each year. For 1958, this number was: 200,259,799,740. The *monthly number* was obtained by dividing the number of cases of the months into the conversion number. Not more than three figures were entered in the table. The figures for these two years are shown on Table VIII.

The monthly number of positive responses to allergenic extracts may be multiplied by the corresponding conversion number. Not more than the first three figures of this product are entered again. The fairly gross error which might be committed as a result of the so-called 'silent' months, is avoided wherever possible by this method.

The numbers for the total incidence of positive tests and, separately, for the fungi as listed under I and II [1], for house allergens and for grass and rye pollens now are:

[1] Fungi I and II: this choice is based on studies by van der Werff. [44]
Fungi I: Mucor racemosus Fres., Rhizopus nigricans Ehrenberg, Penicillium expansum (Link) Thom, Mucor spinosus van Tieghem.
Fungi II: Cladosporium herbarum Link ex. Fr., Trichoderma viride Pers. ex. Fr., Fusarium culmorum (W.Sm.) Sacc., Penicillium chrysogenum Thom, Stemphylium botryosum Wallr., Aspergillus versicolor (Vuill.) Tiraboschi, Botrytis cinerea Pers.

TABLE VIII

Month	1957		1958	
	Number of cases	conversion number	Number of cases	conversion number
January	57	88	34	59
February	39	128	33	61
March	17	293	22	91
April	14	356	14	143
May	13	384	11	182
June	13	384	19	105
July	13	384	20	100
August	22	227	17	118
September	24	208	31	65
October	27	185	26	77
November	22	227	36	56
December	39	128	37	54

	Fungi I				*Fungi II*			
	1957		1958		1957		1958	
	tot. +	ratio	tot. +	ratio	tot. +	ratio	tot. +	ratio
January	8	70	10	59	13	114	8	47
February	13	166	3	18	11	141	7	43
March	3	88	3	27	3	88	2	18
April	3	107	4	57	2	71	1	14
May	6	230	1	18	1	38	0	0
June	7	269	4	42	5	192	2	21
July	7	269	10	100	2	77	7	70
August	6	136	8	94	2	45	8	94
September	10	208	13	85	8	166	8	52
October	14	315	21	162	9	167	15	116
November	13	363	11	62	8	182	8	45
December	10	128	8	43	7	90	3	16

	Grass pollen				*Rye pollen*			
	1957		1958		1957		1958	
	tot. +	ratio	tot. +	ratio	tot. +	ratio	tot. +	ratio
January	3	26	2	12	2	18	2	12
February	7	90	6	37	3	38	5	31
March	1	29	4	36	0	0	3	27
April	2	71	2	29	2	71	2	29
May	1	38	0	0	1	38	0	0
June	4	154	2	21	1	38	1	11
July	5	192	4	40	3	115	2	20
August	3	68	3	35	2	45	4	47
September	1	21	3	20	1	21	1	7
October	0	0	5	39	1	19	3	23
November	2	45	1	6	0	0	1	6
December	2	26	1	5	1	13	0	0

	House allergens				*Total incidence of positive tests*			
	1957		1958		1957		1958	
	tot. +	ratio	tot. +	ratio	tot. +	ratio	tot. +	ratio
January	14	123	18	106	171	150	167	99
February	17	218	14	85	220	282	140	85
March	3	88	11	100	124	363	139	126
April	4	142	8	114	57	203	88	126
May	8	307	2	36	85	326	108	196
June	12	461	11	116	148	568	132	139
July	12	461	12	120	161	618	172	172
August	16	363	9	106	178	404	184	217
September	20	416	19	124	204	424	170	111
October	11	204	17	108	224	414	243	187
November	17	386	16	90	230	522	209	117
December	24	307	8	43	212	271	126	68

A striking feature consists in the fact that conversion shows the incidence of positive reactions to be highest in the second half of the year. The high incidence of positive reactions to fungi probably is due to the peak attained by the number of air-borne spores, the maximum concentration beginning about August and ending in the latter half of November. This also accounts for the two peaks reached by house allergens in September, as the growth of fungi is a constant feature in a very large number of houses in the Netherlands owing to the low soil-water level in this country.

The two peaks shown by house allergens in June and July in 1957 and 1958 cannot merely be accounted for by a reaction to spring-cleaning in the Netherlands; they probably are also due in part to house fungi and the natural break-down products of the latter.

Reactions to food allergens vary markedly. Of the various kinds of fruit, only cherries show a small peak in June both in 1957 and in 1958.

The incidence of positive reactions to grass and rye pollens shows typical variations. As shown in the annual tables, the 'principal season' of the two pollens occurred later in 1958 than it did in 1957; this is attributable to the fact that harvesting was done at a somewhat later period, all the pollen becoming air-borne at a later time of year owing to the unfavourable weather. Especially the grass pollens show peaks in February 1957 and March 1958. These peaks can probably be accounted for by the appearance of several new patients with hay fever in the early part of the year; these patients, having had attacks during the past year (or years), then come to the out-patient department in the hope that they will be protected by desensitization during the coming 'season'.

In order to ensure more reliable data, this investigation would undoubtedly have to be extended to a greater number of years.

4) In conclusion, the results of skin testing with extracts of *seeds*, so-called 'propagative agents', were studied, especially in patients with pollinosis.

In addition to routine skin tests, tests with 20 propagative extracts (cf. p. 84) were also performed in 12 patients showing typical sensitivity to pollen. Reactions were only doubtful in 3 cases. The responses shown by 2 subjects in the scratch test were ++, so that intracutaneous tests were not performed in these cases. The reactions in testing with these propagative extracts usually are more intensive in cases of hay fever than they are in patients with other forms of sensitization. These tests were not done again in the hay fever season.

Conclusion: Not only daily and seasonal variations in the reactivity of the skin, but also the increased sensitivity of the patient to other allergens during the season in which his principal allergen causes allergic manifestations should be taken into account in skin testing.

CHAPTER V

THE PRAUSNITZ-KÜSTNER TEST IN ALLERGY

Prausnitz and Küstner described the passive transfer test named after these authors in 1921. In this test, the serum of a specifically allergic subject is injected into the skin of a normal individual, the specific allergen being injected at the same site 24 hours later. When allergic antibodies are present, passive sensitization of the injected site will result and the allergen will produce an immediate reaction.

Accordingly, this test is direct evidence of the presence of circulating specific antibodies.

As this test is only slightly less sensitive than intradermal testing in the allergic patient himself, this method may be used for testing in allergy without the patient having to be subjected to cuts or injections. Especially in young children, it may occasionally be advisable to withdraw about 5 ml. of blood and to use this in all the tests that have to be made, serum being obtained from the remaining blood in order to produce passive transfer sites in one of the parents. As the production of each site only requires 0.1 ml., the number of sites obtainable with 2 ml. of serum will be sufficient for a complete series of tests. Hansen [4] states that the Prausnitz-Küstner test is microscopically identical with the direct positive intracutaneous test.

A drawback to this method consists in the fact that virus diseases such as homologous serum jaundice or syphilis may be transmitted. Therefore, only well-informed volunteers, preferably relatives, should be selected for this test.

In addition, testing should be preceded by accurate history-taking as well as the Venerial Diseases Laboratory Reaction. If the serum of allergic patients is to be stored, it should be withdrawn under strictly sterile conditions. Haemolysis may be prevented by using a dry syringe. No preservatives having been added, the sterile tube is immediately centrifuged and the serum injected into small 1 ml. ampoules. The ampoules then are immediately sealed up.

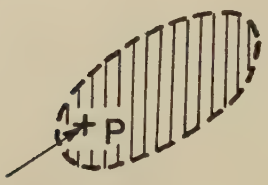
The filled ampoules should be stored at room temperature (18^0—20^0) for three days and examined for clearness. The slightest sign of turbidity in an ampoule should be regarded as possible evidence of infection. In that event, the ampoule should be destroyed. The ampoules that have continued clear are stored in the refrigerator. When an ampoule has been opened for testing, it should be destroyed after use, even if not all the serum has been utilized.

The injections of serum and allergens should always be made at the

same site. Subsequent injections made *beside* the needle puncture may produce smaller reactions. Control tests with the same allergen beside the previously injected site will be negative.

Now this 'injecting at the same site' is an ill-defined notion, to which Klein [26] objected in 1942.

The injection of allergic serum into the opening X in a previously normal skin is followed by the appearance of a sensitized area of a particular form and size, varying with the resistance of the tissues, the rate of injection and the direction in which the injection is made. When the direction of the injection and the needle puncture are not identical, only a part of the sensitized area will come into contact with allergens. The best reaction is produced by injecting in the same direction and into the same puncture.



Direction of needle

Klein was able to ensure a high degree of accuracy by injecting in exactly the same direction and into exactly the same puncture. He then compared his own tests with those made by Cooke. Cooke more or less trusted to luck in making another injection at the previously injected site. Klein marked the previously injected site with carbolfuchsin and also indicated the direction in which the injection should be made. His tests clearly demonstrated that the number of reagins present in the skin following accurate injection was superior to that present on inaccurate injection of an allergen.

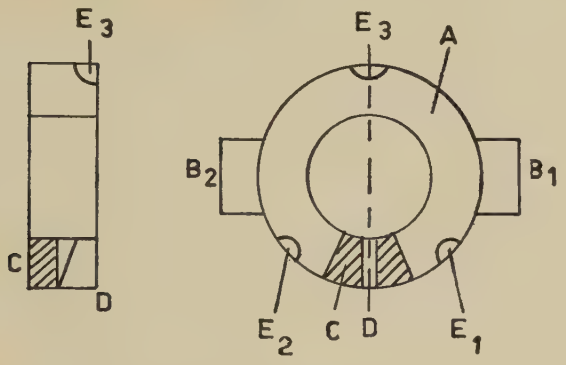
Personal investigations

This method of injection was found to be susceptible of improvement when a simple appliance was used.

A plastic ring (A) was slightly reinforced at the sides to enable compression (B). A notch (C) was made on one side and a round groove slanting inwards (D) was ground in this notch so that a needle will always have to be inserted into the skin at the centre of the circle. Slight concavities were made in three places (E) on the external underside of the ring, reducing the pressure at these points.

The Prausnitz-Küstner test then was performed as follows:

The skin having been cleansed, it is slightly strectched with the right hand and the ring is placed on the test site with the left hand, compression being exerted upon the broad parts B_1 and B_2 by two fingers. The needle is inserted into the groove with the right hand so that the bevel does not touch the side of the ring. Guided by the 'slant', the needle is inserted into the skin with the bevel up, to a point where the opening of the bevel is no longer visible and 0.1 ml. of serum is injected.



The needle then is withdrawn from the skin and compression is continued for another 3 minutes. All reagins now are concentrated within the opening of the circle and will remain fixed in this area. When compression is discontinued, the concavities (E) are immediately recognizable. To keep them visible for a short period, these areas may be painted with carbolfuchsin; when they are lightly touched with a toothpick dipped in silver nitrate, the areas will continue to be identifiable for as long as a week, especially when a small amount of developer is applied as well. This treatment should be carried out very carefully, using small quantities, lest some temporary damage be caused to the skin.

The compressor may be placed on the skin in the same manner 24 hours later and the allergen injected again, the needle being guided by the groove. As a result, all reagins will be concentrated on an accurately defined area of the skin and the allergen will be injected exactly into this site.

This is proved by the following experiment:

A compressor having a triangular and another having a starshaped cavity were made instead of a ring. Another Prausnitz-Küstner test was performed using this appliance. The area of redness also assumed the form of a triangle and a star respectively following the second injection.

This method of performing the Prausnitz-Küstner test was compared with Klein's method. The tests were made on a number of volunteers.

Evidence that the compression method is actually superior to the other form of testing might be provided by repeating the tests; in that event, the results obtained in the personal test should always be the same and those obtained using Klein's method should vary. In order to decide whether this was so, 0.1 ml. of grass pollen serum was injected three times in 2 volunteers, the injections being made at the same level of the upper arm and placed 5 cm. apart. The tests using compression were always made on the right upper arm, Klein's method being used on the left upper arm.

TABLE IX

A) Serum of patients hypersensitive to grass pollen

	Personal method			*Klein*		
	Using compression			Without compression		
	1.000 U	100 U	10 U	1.000 U	100 U	10 U
1	++±	+±	—	++	+	—
2	++	+	±	++	±	—
3	+++±	++	±	+++	++	—
4	++	+	—	+	±	—
5	++	+	—	++	+	±
6	++	+	±	++±	+	—
7	++±	+	—	++±	+±	±
	16½	8½	1½	15	7½	1

B) Serum of patients hypersensitive to shrimps

	Using compression				Without compression			
	5 %	1 %	½ %	$\frac{1}{10}$ %	5 %	1 %	½ %	$\frac{1}{10}$ %
1	++	++	+±	+	++	+	+	±
2	+±	+	+	—	+±	+	—	—
	3½	3	2½	1	3½	2	1	½

C) Serum of patients hypersensitive to pea-nuts

	Using compression			Without compression		
	5 %	1 %	$\frac{1}{10}$ %	5 %	1 %	$\frac{1}{10}$ %
1	++	+±	±	+±	+	±

The following day, the sensitized test sites were all injected with 0.02 ml. of 500 units of grass pollen allergenic extract, the test being repeated with 100 units at the same sites a week later. The skin reactions produced were found to be exactly similar in shape and size when compression was used and to be of various forms and sizes in two cases when Klein's method was employed.

Conclusion: Compression is the method of choice in verifying whether a skin reaction is specifically positive or due to non-specific side-effects in doubtful cases.

Moreover, it is the best method in all qualitative and quantitative determinations concerning allergens and reagins in vivo, to be made in the course of scientific investigations.

CHAPTER VI

PROBLEMS RELATING TO THE STERILIZATION AND PRESERVATION OF ALLERGENS

§ 1) *Sterilization of allergens*

Sterilization is carried out for the purpose of destroying a maximum number of pathogenic organisms present in the extracts. In doing so, measures should be taken to prevent the extracts from causing non-specific irritation and to avoid reducing the potency. In actual practice, allergens may be sterilized by:

A) filtration,
B) heat,
C) chemical sterilization.

A) Filtration

This is a very widely used method. The extracts may be passed through a Berkefeld filter, a Jena glass filter (the C 5 bacteria - tight filter) or a Seitz filter. Each time the extract is passed through a filter, there will be some loss of allergens.

Ebel [20] studied the loss of allergens resulting from the passing of extracts through Berkefeld filters by determining the nitrogen content. Ebel concluded that the passing of alcoholic extracts through filters causes the greatest loss, viz., about 50 per cent.

The loss of allergens will depend on the size of the pores in passing extracts through a Berkefeld filter.

B) Sterilization by heat

The literature contains occasional references to the possibility and advisability of sterilizing allergens by heat. As most allergens are assumed to have a protein structure and as the structure of proteins is altered by heat, it may well be that their allergenic properties are lost or at any rate considerably altered. If the allergens should be found to be thermostabile, then sterilization by boiling would be possible. This would be a great advantage, especially in the case of commercial allergenic extracts. Wells and Osborne [43] write: 'The sensitivity produced by cooked foods is in no way comparable to that generally established with the raw material.' This is confirmed by Furth [22].

Ratner and Gruel [34] state that the antigenic action of certain proteins is reduced by heat. They believe this to vary with the thermostability or lability of the molecular constituents and the duration of heating.

Kate Maunsell [7], however, heated fungus spores at 100° on three

consecutive days without any demonstrable decrease in potency resulting. Accordingly, the question whether there are allergens that can be sterilized, may be answered in the affirmative. It is doubtful, however, whether *all* allergens will not be affected by heat.

TABLE X

Grass pollen 10.000 U

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	++	++++	++++	+++
2	male	++	+++	+++	+++
3	male	+++	++++	++++	++++
4	male	+++	+++	+++	+++
5	female	+++	+++	+++	++++
Total		13	17	17	17

Grass pollen 1.000 U

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	+++	+++	+++	++
2	male	+±	++	+++	++++
3	male	++	++	++	++
4	male	++++	+++	+++	++
5	male	++	+	++	+
6	male	+	++±	+++	++
7	male	++	+++	+++	++++
8	male	+++	+++	+++	+++
9	male	+	++±	++±	++
10	male	—	—	+	++
11	female	+±	+	+	+
12	female	±	+±	++±	++
13	female	++	++	+++	+++
14	female	++	++	++	++
15	female	++	+++	+++	++++
Total		27½	31½	37	36

Grass pollen 100 U

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	female	—	—	—	+
2	female	++	++	++	++
3	female	++	++	+++	+++
4	female	++	++	++	++
5	female	++	+	++	+
6	female	++	+	+	++
7	male	±	+	++	+++
8	male	++	++	++	++
9	male	+++	++	++	++
10	male	++	++	+±	+±
11	male	+	—	—	—
12	male	—	+	+	±
13	male	—	+	—	—
14	male	—	—	—	+
Total		18½	17	18½	21

Grass pollen 10 U

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	±	±	+	+±
2	male	++++	++	+	+
3	male	+	+	—	—
4	male	±	—	±	+
5	male	++	++	+	±
6	female	+	+	++	++
7	female	+	+	+	+
8	female	++	±	+±	++
9	female	±	±	±	+
Total		12½	8½	8½	10

5 % extract of chicken feathers

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	+++	+++	+++	++
2	male	++	++	++	++
3	male	+±	+±	+±	++±
4	female	++	+++	++±	+++
5	female	++	+++	+++	+++±
Total		10½	12½	12	13

1 % extract of chicken feathers

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	+	+	+±	+±
2	male	++	+±	++	++
3	male	+±	+±	++	+±
4	male	++	+±	++	+±
5	female	++	+±	+±	+±
6	female	++	++±	++±	++±
7	female	+±	+±	++	+±
8	female	+++	++	+	+++±
Total		15	13	14½	15½

5 % protein

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	—	—	+	+
2	male	—	—	±	+
3	male	±	+	+	+
4	female	—	—	+	++
5	female	—	+	+±	—
6	female	+	+	++	+±
7	female	+++	+++	++	+
8	female	±	+	++	++
Total		5	7	11	9½

Shrimps 1 %

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	+	+	+	+++
2	male	+±	++	+±	+
Total		2½	3	2½	4

Egg yolk 5 %

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	—	+±	+±	—
2	male	—	—	—	+
3	male	++	++	+±	+
4	female	±	±	+	+±
5	female	+±	+±	++	+
6	female	±	—	+	+±
7	female	+	+	±	±
8	female	+	+	±	+
Total		6½	7½	8	7½

Flounder (lean fish) 5 %

Case	m./f.	15°	5′ 60°	5′ 80°	5′ 100°	30′ 100°
1	male	+	+	+	++	+±
2	male	+±	+±	++	+++	++
3	male	+++	+++	+++	+++	+++
4	female	±	±	+	±	±
Total		6	6	7	8½	7

Herring (fat fish) 5 %

Case	m./f.	15°	5′ 60°	5′ 80°	5′ 100°	30′ 100°
1	male	±	±	+	+	±
2	male	+	±	+	+	—
3	male	+	±	±	±	+
4	male	+++	+	—	±	—
5	male	+	++	+±	+±	++
6	female	+	+	+	++	+
Total		7½	5½	5	6½	4½

Eel 5 %

Case	m./f.	15°	5′ 60°	5′ 100°	30′ 100°
1	male	±	+	+	+
2	male	++±	++	+++	++
3	male	—	±	±	±
4	female	+	+	±	+
Total		4	4½	5	4½

Personal investigations

a) In order to study the effects of the temperature and the duration of heating, extracts of grass pollen, chicken feathers, protein, egg yolk, flounder (lean fish), herring (fat fish), shrimps and eel were prepared at 15°; a number of extracts were then heated at 60° for 5 minutes, others were boiled at 100° for 5 minutes and others again were boiled at 100° for 30 minutes; the fish extracts were also boiled at 80° for 5 minutes.

The results obtained in testing are shown in Table X. For the sake of convenience, the results were added to enable a comparison.

Only *those* tests were listed, in which control injections were negative and tests with 1 : 10.000 histamine were positive.

These investigations show that all allergens are virtually thermostabile up to 100°. Only protein, egg yolk, flounder and herring were found to have slightly decreased in potency following 30 minutes boiling.

b) These findings raise the question of whether the allergen will be inactivated by prolonged boiling and boiling at higher temperatures.

Therefore:

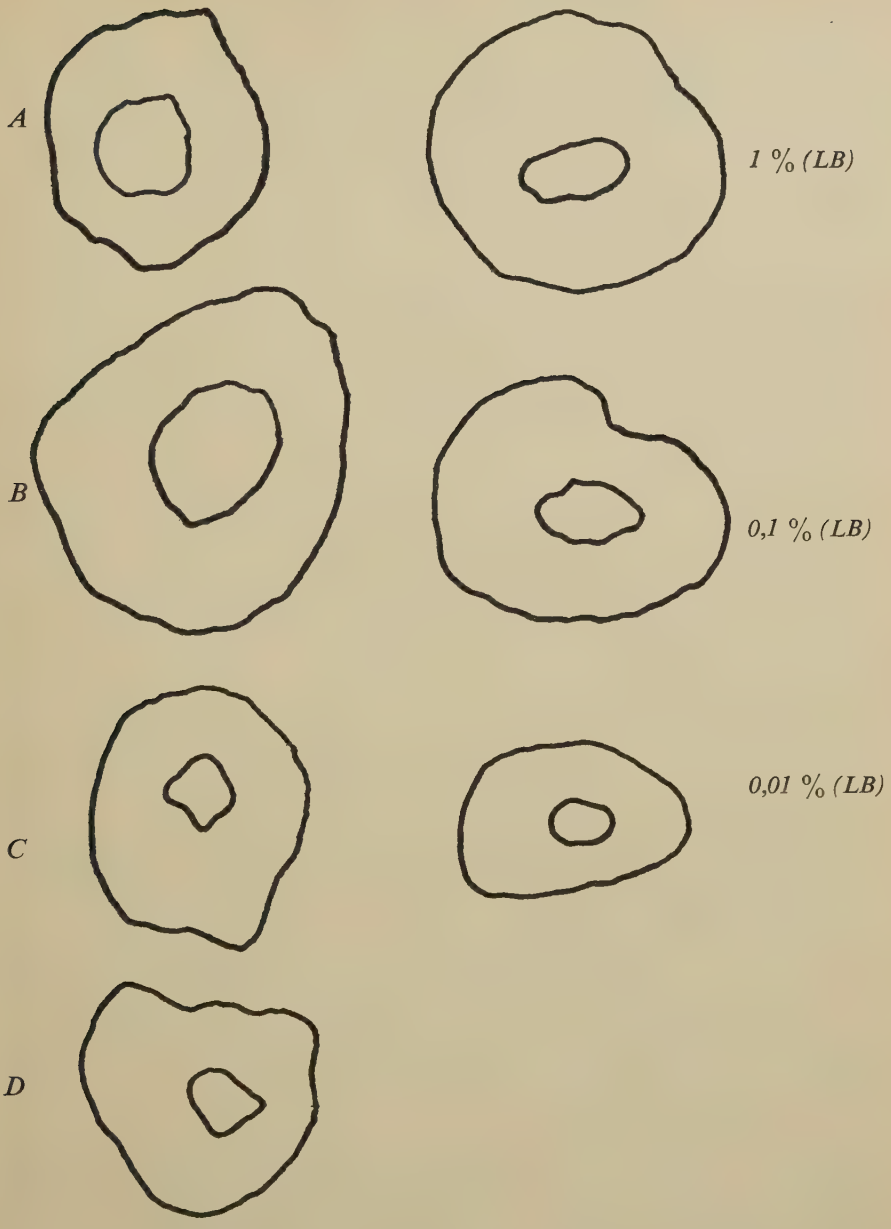
1) an allergen was boiled for 30 minutes on 3 consecutive days,

2) an allergen was subjected to prolonged boiling,

3) an allergen was boiled at temperatures above 100°.

When boiled as described under *1)*, the allergenic action was not found to be altered.

When extracts were boiled for 2, 4, 6 and 8 hours, both the intracutaneous test and the Prausnitz-Küstner test with house dust and grass pollen were found to produce responses approximately identical with the initial reaction if boiled up to 2 hours; when the extracts had been boiled for 4 hours, the reactions were markedly smaller, being less than



Results of the Prausnitz-Küstner test with housedust, when the extracts were boiled for 2 (B), 4 (C), 6 (A) and 8 (D) hours, compared with those obtained with the housedust extract used in the allergy outpatient department of the O.-R.-L. department of the University Hospital of Leyden (LB) in one percent, 0,1 percent and 0,01 percent dilutions.
The order A, B, C and D was adopted by R. Voorhorst who performed this test at the request of the present author, to check the results obtained in testing by the latter, as the periods of boiling had not been disclosed to him.

one-tenth of the initial reaction; the reactions produced by extracts subjected to prolonged boiling, however, did not become any smaller. On heating up to 180° for 30 minutes, the reactions were reduced to from one-tenth to one hundredth of the initial value; on heating at very high temperatures (30 minutes at 300°), reactions to house dust and fish extracts were found to have become negative. This high temperature was attained by heating at 65 atmospheres of overpressure.

C) Chemical sterilization and filtration

Allergenic extracts cannot only be sterilized by boiling and passing the extracts through filters, the pores of which have a particular width and through which liquids and small corpuscles can pass, whereas bacteria are retained (Seitz, Berkefeld and Chamberland filters), but also by chemicals.

Personal investigations

The results obtained in intracutaneous testing with allergenic extracts sterilized by various methods were compared:

a) allergens sterilized by passing the extracts through a Seitz filter;
b) allergens sterilized by fractional boiling of the extracts for 30 minutes on 3 consecutive days;
c) allergens sterilized by the addition of 0.5 per cent phenol to the extracts.

The extracts treated by these methods were injected into 1 ml. ampoules which were sealed up and stored in the refrigerator three days later after the extracts had been examined for turbidity. Each ampoule was used only once in testing. When the ampoule was opened, the allergenic solution was immediately drawn into a dry sterile syringe; the ampoule was destroyed after testing.

These tests were performed in patients in whom testing was indicated anyhow in view of their condition. Besides injections of allergens, control tests with 1 : 10.000 histamine, physiologic saline and Coca's solution were made in every patient. All trial injections were made on the lateral surface of the forearm, the control injections being made at the same level on the medial surface.

1) Thirty patients were tested with grass pollen, 30 with house dust allergens, 3 with egg, 3 with coffee, 3 with cocoa, 3 with nescafé, 4 with feathers, 3 with nuts, 8 with Penicillium brevis compactum, 8 with Mucor racemosus, 3 with mixed flour, 3 with banana, 7 with pork and 3 with strawberry.

These investigations show [Table XI] *that all allergens can be sterilized, either by the addition of phenol or by passing the extracts through a Seitz filter.* When the latter method is used, many allergens are lost, however, so that the results obtained in skin testing with these extracts will be

TABLE XI

Grass pollen 100 U			House dust extract 1 %			Pork 1 %		
Case	Seitz	Sterile	Case	Seitz	Sterile	Case	Seitz	Sterile
1	++	+	1	++	++	1	++	++++
2	+	++	2	+	+±	2	++	++
3	++	++	3	+±	+±	3	+	±
4	+±	+++	4	++	++	4	+±	++
5	+	++	5	++	++	5	++	++
6	±	±	6	++±	+++	6	+	±
7	+	+±	7	+	+±	7	+	±
8	+	+±	8	++	++			
9	++	+++	9	++	+++			
10	++	+++	10	±	++			
Total	14	19½	Total	16½	20½	Total	10½	11½

Egg 1 %			Nescafe 1 %			Mixed flour 1 %		
Case	Seitz	Sterile	Case	Seitz	Sterile	Case	Seitz	Sterile
1	++	±	1	++	++	1	++	+++
2	±	±	2	+	+	2	+	++±
3	±	+	3	±	+±	3	++	++
Total	3	2	Total	3½	4½	Total	5	7½

Cocoa 1 %			Coffee 1 %			Strawberry 1 %		
Case	Seitz	Sterile	Case	Seitz	Sterile	Case	Seitz	Sterile
1	+	+	1	+++	++	1	+±	+±
2	+	+++	2	+	+	2	±	++
3	—	—	3	±	+±	3	+	+
Total	2	4	Total	4½	4½	Total	3	4½

Feathers 1 %			Nuts 1 %			Banana 1 %		
Case	Seitz	Sterile	Case	Seitz	Sterile	Case	Seitz	Sterile
1	+	+++	1	+	++	1	++	+++
2	++±	++++±	2	+++	++	2	+±	+±
3	+	++	3	+	±			
4	++	++						
Total	6½	11½	Total	5	4½	Total	3½	4½

Penicillium brev. comp. 1 %			Mucor racem. 1 %		
Case	Seitz	Sterile	Case	Seitz	Sterile
1	±	+	1	±	±
2	±	++	2	+	±
3	+±	+++	3	+±	+±
4	±	±	4	±	+
5	+±	+±	5	+±	+±
6	+++	+++	6	+	+
7	+	+	7	+	+
8	+	+	8	+	±
Total	9½	13	Total	8	7½

Ratio skin tests Seitz : sterile = 80 %.

Maize pollen 250 U

Case	Coca	Sterile
1	+±	++
2	++	+++
3	++	++
4	++	++±
5	++	+±
6	+++	+++
7	±	+±
8	+	++
9	+++	++++
10	+±	+±
11	++	++
12	++	+±
13	++	+±
14	+±	++
15	++	++±
Total	28	32½

House dust extract 1 %			Grass pollen 100 U		
Case	Coca's solution	Phenol ½ %	Case	Coca's solution	Phenol ½ %
1	++	+±	1	+	++
2	+±	++	2	++	+
3	++±	++±	3	++±	++
4	+±	++	4	+	+
5	++	++	5	++±	++
6	++	++	6	++	++
7	+±	+±	7	+	±
8	+++	++±	8	++	+±
9	++	++	9	+	+
10	++	++	10	+	+
			11	+±	+
			12	+±	+±
	20	20		19	16½

Pork 1 %			Grass pollen 100 U			Feathers 1 %		
Case	Coca's solution	Sterile	Case	Coca's solution	Sterile	Case	Coca's solution	Sterile
1	++±	++++	1	++	+++	1	+	+±
2	++	++	2	++	++	2	++	+±
3	+±	+±	3	+±	++	3	+±	++±
4	+±	++	4	++	+±	4	+±	++
5	++	++	5	—	+	5	++±	+±
6	+±	+±	6	+	+			
7	+±	+						
Total	12½	14	Total	8½	10½	Total	8½	9

Grass pollen 250 U				House dust 1 %			
Case	Seitz	Sterile	C5 glass	Case	Seitz	Sterile	C5 glass
1	++	++	+±	1	++	++±	++
2	+	++	++	2	+	++	++
3	++	+±	++	3	++	+±	+
4	+±	++	+	4	++	++	++
5	++	++	+±	5	+	+±	±
6	+	±	—	6	+±	++	+++
7	—	+	++	7	+	+	++
8	+	+±	++	8	±	+	++
9	+±	++	+	9	++	++±	+±
10	++	++	+±	10	+	++	+±
11	+	++	++	11	+++	++±	++
12	+	±	—	12	+	+±	++
13	—	±	++	13	++	+±	++
14	++	++	+	14	+	++	+
15	+	±	+±	15	+±	++±	++±
16	+	+	±	16	+	±	—
17	++	+++	++	17	+±	++±	++
18	+	++	++	18	++	+++±	+±
19	+	+±	++±	19	+	+±	++±
20	+	++	+	20	+	+	+±
Total	25	31½	29	Total	29	37	34½

found to be about 20 per cent smaller when the plus-signs are added up and compared with those obtained using the sterilized extracts. Accordingly, there is a considerable loss of allergens; it is not permissible, however, to conclude that there is a corresponding proportional loss of allergens by comparing the size of the wheals. Quantitative determinations can only be made with the Prausnitz-Küstner test.

2) Tests with an extract to which Coca's solution was added and with an extract sterilized by boiling, which tests were performed with maize pollen (15 cases), pork (7 cases) and feathers (5 cases), turned out in in favour of boiling, the difference being very slight.

The addition of 0.5 per cent phenol does result in a slight loss of potency (viz., about 6 per cent of the skin reaction).

3) Tests with extracts sterilized by passing them through a Jena glass filter (C 5), by passing them through a Seitz filter and by boiling

showed only a very slight difference in favour of boiling in 20 cases of house dust allergy and 20 of grass pollen allergy.

4) Follow-up studies were done in the Allergy Out-Patient Department of the Hospital for Diseases of the Ear, Nose and Throat of the University of Leyden (see page facing).

Grass pollen extracts of 250 units and 1 per cent house dust extracts were compared following 4 methods of sterilization, viz.:

a) boiling,
b) chemical sterilization (Coca's solution),
c) passing the extract through a Seitz filter,
d) passing the extract through a Jena C 5 filter.

The accompanying diagram shows the results obtained in the Prausnitz-Küstner test with a 1 per cent house dust extract prepared by the above methods. The 1 per cent Lifa extract which was also passed through a glass filter bears a fairly close resemblance to the personal extract following its sterilization by passing it through a Jena glass filter.

Conclusion: Not only comparative skin tests, but especially passive transfer tests clearly show that the skin reactions produced by extracts passed through a Seitz filter are much smaller!

§ 2) *Ensuring the sterility of solutions of allergens which are in constant use*

One of the chief problems is how to keep allergenic extracts which are in constant use free from bacteria.

Baumann [14] states the following factors as causes of infection:

1) the material itself contains bacteria;
2) when the needle is inserted into the bottle, it is infected by contact with the finger or by micro-organisms on the cap of the bottle or the neck of the ampoule, e.g. as a result of filing through the neck.

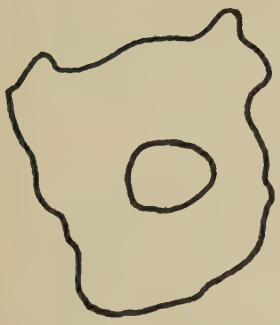
Regamey, Thomann and Dedie [14] showed that fifty per cent of the needles were no longer free from bacteria after they had been used once in making intradermal injections.

Personal investigations

1) In order to determine the period for which allergenic extracts will remain sterile when they are in normal use, bottles were filled with 5 ml. of a 1 per cent sterile pork extract, a 1 per cent extract of house dust and 100 Units of grass pollen extract, the bottles then being sealed with a rubber cap.

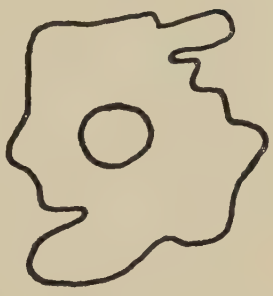
One series of these 3 extracts was stored in the refrigerator at — 4° C., another series being stored at room temperature.

All extracts contained 0.5 per cent phenol. The caps having been cleaned with spirit, a dry sterile needle was pricked through the cap three times a week, the needle was brought into contact with the



I

Prepared by usual method and chemically sterilized. *Personal extract.*



II

Prepared and sterilized by usual methods. *Personal extract.*

III

Passed through C5 Jena bacteria-tight filter after extraction. *Personal extract.*

IV

Passed through Seitz filter after extraction. *Personal extract.*

V

Passed through C5 Jena bacteria-tight filter after extraction. *Lifa extract.*

House dust extract was injected
(1 % personal extract of house dust and 1 % Lifa extract of house dust)

solution by turning the bottle and it was withdrawn again after 5 seconds.

The bottles stored in the refrigerator were rapidly unfrozen prior to use and returned to the refrigerator 3 hours later. This length of time was chosen, as in actual practice allergens remain at room temperature during surgery hours.

A drop of liquid was withdrawn from the bottles, placed on a slide, stained with methylene blue and examined microscopically every fourteen days.

The periods elapsing until demonstrable infection had appeared in the bottles stored at room temperature, were:

1) pork extract	bottle 1: 8 weeks bottle 2: 10 weeks bottle 3: 10 weeks
2) house dust extract	bottle 1: 10 weeks bottle 2: 12 weeks bottle 3: 14 weeks
3) grass pollen extract	bottle 1: 10 weeks bottle 2: 14 weeks bottle 3: 18 weeks

The periods elapsing until the conset of infection in the bottles stored in the refrigerator were:

1) pork extract	bottle 1: 12 weeks bottle 2: 12 weeks bottle 3: 14 weeks
2) house dust extract	bottle 1: 12 weeks bottle 2: 14 weeks bottle 3: 18 weeks
3) grass pollen extract	bottle 1: 14 weeks bottle 2: 16 weeks bottle 3: 18 weeks

This shows that the onset of the growth of bacteria in the bottles stored at room temperature usually occurs some weeks earlier than it does in the extracts stored in the refrigerator.

Accordingly, allergenic extracts used only once three times a week will no longer be reliable after from 8 to 12 weeks. This means that, despite all precautions, the number of bacteria introduced into the solution by thrusting the needle thirty times through the rubber cap is sufficient to cause massive growth in spite of the addition of 0.5 per cent phenol and storage in the refrigerator.

Conclusion: It is essential that extracts used 20 *times should be destroyed or that the extracts should be boiled for* 30 *minutes every month.*

Therefore, the quantities of extract used in desensitization should not be more than 10 *ml. per bottle for each patient.*

2) The question was put whether the pH is a factor in the growth of bacteria in extracts. Therefore, solutions of house dust and feathers having pH levels of 6.5, 7.5 and 8.5 were prepared by Menghini's [31] method. These extracts were stored in the refrigerator and given the same treatment as the above series.

Though there is an optimum growth of bacteria at a certain pH, the pH levels used were not found to have any material effect in inhibiting the growth of bacteria.

3) In regard to the preservation of allergens it also is of importance to know whether a particular *pH* is characterized by an *optimum potency* of the extract and whether variations in acidity or alkalinity will affect the skin reactions. For it might be that the pH of the extracts, and consequently their potency, is altered during storage in the refrigerator.

Buffer solutions were prepared as specified by Menghini in the dispensary of the Leyden University Hospital.

The buffer solutions prepared on September 15, 1956 and November 12, 1956 were found to have the following pH levels:

1. Sept. 15, 1956: pH 6.54, pH 7.56 and pH 9.10 respectively,
2. Nov. 12, 1956: pH 6.50, pH 7.55 and pH 9.10 respectively.

The pH of the most alkaline buffer solutions fell to 8.5 within a week. The pH subsequently only showed a slow decline. When an examination was made 5 weeks later, the pH of the September buffer group was found to be 8.3, that of the other group being 8.5. Since alkalization by glass only occurs on heating of the glass, as observed by Esty and Catheart (cf. p. 27), this contingency did not have to be taken into account.

The question was put whether the pH of allergenic extracts stored in the refrigerator in 10 ml. rubber-stoppered bottles will remain at a constant level.

Extracts having a pH of 6.5, 7.5 and 8.5 were prepared from the food allergens and inhalants listed below and stored in the refrigerator.

The pH was determined on December 4, 1956, January 10, 1957 and March 1, 1957.

These observations show that the pH decreases considerably within a few months in some cases, the average decrease being from 6.5 to 6, from 7.5 to 6.8 and from 8.5 to 7.4 c[f. Table XII].

This decline usually proceeds at a slower rate in inhalants, the average decrease being from 6.5 to 6.4, from 7.5 to 7.1 and from 8.5 to 8.0.

TABLE XII

Banana	4/12 — 6.5	10/1 — 6.2	1/3 — 5.2
	4/12 — 7.5	10/1 — 7.0	1/3 — 6.4
	4/12 — 8.5	10/1 — 8.2	1/3 — 7.2
Cocoa	4/12 — 6.5	10/1 — 6.5	1/3 — 6.4
	4/12 — 7.5	10/1 — 7.2	1/3 — 6.8
	4/12 — 8.5	10/1 — 8.2	1/3 — 7.2
Coffee	4/12 — 6.5	10/1 — 6.1	1/3 — 5.7
	4/12 — 7.5	10/1 — 7.2	1/3 — 6.4
	4/12 — 8.5	10/1 — 8.2	1/3 — 6.4
Nescafé	4/12 — 6.5	10/1 — 6.1	1/3 — 5.4
	4/12 — 7.5	10/1 — 7.1	1/3 — 5.7
	4/12 — 8.5	10/1 — 8.1	1/3 — 6.3
Mixed flour	4/12 — 6.5	10/1 — 6.4	1/3 — 6.2
	4/12 — 7.5	10/1 — 7.5	1/3 — 7.2
	4/12 — 8.5	10/1 — 8.4	1/3 — 8.4
Sauerkraut	4/12 — 6.5	10/1 — 6.0	1/3 — 6.0
	4/12 — 7.5	10/1 — 7.0	1/3 — 6.6
	4/12 — 8.5	10/1 — 8.0	1/3 — 7.0
Pea-nut	4/12 — 6.5	10/1 — 6.3	1/3 — 5.7
	4/12 — 7.5	10/1 — 7.1	1/3 — 6.2
	4/12 — 8.5	10/1 — 8.0	1/3 — 6.2
House dust	4/12 — 6.5	10/1 — 6.5	1/3 — 6.4
	4/12 — 7.5	10/1 — 7.3	1/3 — 7.2
	4/12 — 8.5	10/1 — 8.4	1/3 — 8.4

Feathers	4/12 — 6.5	10/1 — 6.4	1/3 — 6.3
	4/12 — 7.5	10/1 — 7.4	1/3 — 7.2
	4/12 — 8.5	10/1 — 8.3	1/3 — 8.0
Mucor racem.	4/12 — 6.5	10/1 — 6.4	1/3 — 6.4
	4/12 — 7.5	10/1 — 7.3	1/3 — 7.2
	4/12 — 8.5	10/1 — 8.0	1/3 — 7.8
Penic. brev. comp.	4/12 — 6.5	10/1 — 6.5	1/3 — 6.4
	4/12 — 7.5	10/1 — 7.4	1/3 — 7.4
	4/12 — 8.5	10/1 — 8.1	1/3 — 7.8

4) Are there variations in the potency of allergenic extracts when the pH is standardized at different levels?

To answer this question, the following allergenic mixtures were compared:

1) Allergens extracted with Coca's solution and sterilized.
2) Allergens extracted with a buffer solution having pH 6.5 and sterilized.
3) Allergens extracted with a buffer solution having pH 7.5 and sterilized.
4) Allergens extracted with a buffer solution having pH 8.5 and sterilized.

Extraction was done within from 7 to 14 days after preparation of the buffer solutions, i.e. after the solutions had become constant.

In addition, the usual control tests with Coca's solution and the various buffer solutions were performed in every case.

The allergens studied were grass pollen, house dust, egg, cocoa, coffee, nescafé, feathers, nuts, mixed flour, Mucor racemosus, Penicillium brevis compactum, banana, strawberry and melon, solutions having pH 6.2, 7.1 and 7.5 being tested in a patient with apple allergy. Injections of a pollen extract were also made in a number of patients, an extract of house dust being injected in others. The pH levels of the two extracts were 7.1 and 7.4. The results are listed in Table XIII.

These studies show that there are no marked differences of potency between allergenic extracts having pH 6.5, 7.5 and 8.5, but that the optimum effect does occur at about 7.5.

Conclusion: As the potency of the allergens was found to be affected to only a slight extent by the pH, there is no point in preparing allergenic extracts at a particular pH level, nor in adjusting them to a particular pH.

TABLE XIII

Grass pollen 100 U				
Case	Sterile	pH 6½	pH 7½	pH 8½
1	+	±	+	+±
2	++	+±	+++	+++±
3	++	++	++	++
4	+++	++	++±	+++
5	++	+	+	++
6	±	±	++	+
7	+±	+±	+++±	++±
8	+±	±	+++	++
9	+++	+	++	++
10	+++	++	++±	+++
Total	19½	12½	22½	22½

Melon 1 %				
Case	Sterile	pH 6½	pH 7½	pH 8½
1	+	+±	+	+
2	±	++	+	+
3	±	±	±	+±
4	+	+	+±	+
5	±	+±	+	±
Total	3½	6½	5	5

Egg 1 %				
Case	Sterile	pH 6½	pH 7½	pH 8½
1	±	++	+	±
2	±	+±	+±	±
3	+	++	+±	±
Total	2	5½	4	1½

House allergenic extract 1 percent				
Case	Sterile	pH 6½	pH 7½	pH 8½
1	++	++	++	++
2	+±	+	++	++±
3	+±	++	++±	++
4	++	++±	+±	++
5	++	++±	+++±	++±
6	+++	++±	++±	+++
7	+±	+±	+	+±
8	++	++	++	+±
9	+++	+++	+++	++
10	++	—	++	++
11		++	+++	+++
12		+++	+++	++±
13		+++	+++±	++±
14		++	++±	++
15		++	++	++
16		+++±	++	+±
17		++±	+++	+++±
18		+++	+++	++
19		++	++	++
Total 1/10	20½	19	22	21
Total 11/19		23	24	21
Total 1/19		42	46	42

Strawberry 1 %				
Case	Sterile	pH 6½	pH 7½	pH 8½
1	+±	+±	++±	++
2	±	++	+	+
3	+	+	+	+
Total	3	4½	4½	4

Mixed flour 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	++	+±	+++	++
2	++	++	++	++
3	++	±	++	++
4	+	+	++±	++
5	+	+	++	+
6	+±	++	++±	++
7	++±	+++	+++	++
8	+	+	+±	+
9	++	+++	+++	++±
10	+±	+	+	±
11	++	++	+±	+±
12	+±	++	++	++
Total	20	20	26	20½

Nescafe 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	++	++	++	+
2	+	++++	++	++
3	+±	+	+±	+
Total	4½	7	5½	4

Cocoa 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	+	+	+	+
2	++±	++±	++	++
3	+	++±	+±	+±
Total	4½	6	4½	4½

Pork 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	++++	++±	++	++
2	++±	+++	+++	++
3	±	+	+	+
4	±	++	+++	++
5	++	++	++±	+±
6	+	+±	+±	+
7	+±	+	+	±
Total	12	13	14	10

Coffee 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	++	+±	+±	++
2	+	+	+	++
3	+±	+±	++±	++
Total	4½	4	5	6

Apple 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	+±	++	++±	++

Banana 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	+++	+++	+++	++
2	+±	+±	+±	+±
Total	4½	4½	4½	3½

Penic. Brev. Comp. 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	+±	++	+±	+
2	++	++	++	++
3	++±	+++	++++	+++±
4	+	++	++±	++
5	+±	++	+±	++±
6	++	+++	+++±	+++
7	++±	++	++	++
8	+±	++	++±	++
Total	14½	18	19½	18

Mucor Racem. 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	+	+±	++±	+±
2	+±	+±	+	+
3	+±	++	++	+
4	+±	++	++	+
5	±	++	+±	++±
6	+±	+	++±	+±
7	++	+±	++	+
8	++	++	++	++
Total	11½	13½	15½	11½

Feathers 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	+++	+++	++	++
2	+++±	+++	++	+++
3	++±	++	++	+++
4	++	++	++±	++
Total	11	10	8½	10

Pea-nuts 1 %

Case	Sterile	pH 6½	pH 7½	pH 8½
1	++±	+±	+±	+±
2	++±	+++±	+++±	+++ +
3	+	+	++	++
4	++	+++	++++	+++
5	+	++	++	+±
Total	9	11	13	12

1) Extracts	Sterile	pH 6½	pH 7½	pH 8½
Grass pollen	19½	12½	22½	22½
House allergen	20½	19	22	21
Melon	3½	6½	5	5
Egg	2	5½	4	1½
Strawberry	3	4½	4½	4
Mixed flour	20	20	26	20½
Pork	12	13	14	10
Coffee	4½	4	5	6
Nescafé	4½	7	5½	4
Apple	1½	2	2½	2
Penic. Brev. C.	14½	18	19½	18
Mucor. Racem.	11½	13½	15½	11½
Feathers	11	10	8½	10
Nuts	9	11	13	12
Cocoa	4½	6	4½	4½
Banana	4½	4½	4½	3½
Total	146	157	176½	156
2) Other house allergens		23	24	21
Total pH		180	200½	177

Apple 1 %

Case	pH 6.2	pH 7.1	pH 7.5
1	+±	++	++
2	+	+	+±
3	+±	+	+
4	+±	+	±
5	+±	++	++
6	+	+	++
7	++	++±	++±
	10	10½	11½

House allergen 1 %

Case	pH 6.2	pH 7.1	pH 7.5
1	±	+	+
2	+±	++	++
3	++	+±	+±
4	++±	++	++±
5	+±	±	+
6	+±	+++±	+++
7	±	+	+±
8	+++	++	++±
	13	13½	15

House allergen 1 %

Case	pH 7.1	pH 7.4
1	+	+±
2	+	++
3	+	+
4	±	+
5	++	+
6	+	+
7	+±	+
8	++	++
9	±	+
	10½	11½

Grass pollen 100 U

Case	pH 7.1	pH 7.4
1	+±	++
2	±	+
3	+±	++
4	+	+
5	±	+
6	+++	++
7	+±	+
8	±	++
9	++	±
10	+	—
11	+	+
12	+±	++
	15½	15½

CHAPTER VII

RATIONALIZATION OF THE TESTS BY ARRANGING THE ALLERGENS IN GROUPS

In order definitely to establish whether a patient is sensitive to a particular substance, a very large number of tests would have to be performed. To the general practitioner, it is of importance to know which allergens frequently produce positive reactions and which allergens hardly ever give rise to allergy. The allergens may then be arranged in groups according to their pathogenicity.

Some data on the subject are available. As regards the Netherlands, Ten Cate [17] and Franssen [21] have published statistics, the data reported by Ten Cate mainly relating to asthmatic patients and those reported by Franssen only concerning inhalants in patients with vasomotor rhinitis. Accordingly, the two papers are concerned with only some of the allergic diseases and only a part of the allergens. A more detailed paper was written by Alexander [6] who reports the results obtained in skin tests in over 5.000 cases.

Unfortunately, Alexander's statistics are also incomplete, as two of the principal inhalants, viz. fungi and house dust, are not included.

A comparison of these percentages with the findings in 350 personal cases shows that the proportion of positive reactions to the various inhalants and food allergens varies considerably [Table XIV].

This may be due to:

a) the fact that a *constant* contact with allergens is more injurious than contact at periodic intervals (cf. the differences in Ten Cate's table and that of the present author, e.g. as regards hay).

b) the fact that the results of Ten Cate's investigations cannot be entirely identical with the results obtained in personal cases, as Ten Cate only compiled a table relating to asthmatic patients, although the skin reactions to certain allergens undoubtedly are more reliable in cases of urticaria and vasomotor rhinitis than they are in asthmatic patients.

To avoid committing too gross errors, about 7 inhalants and about 21 food allergens should be injected in each case.

The four statistical studies show that the following allergens should be included in a list of the most important inhalants and food allergens: (cf. p. 78)

TABLE XIV

Comparison of the proportion of positive skin tests with the various allergens in the order of incidence in 350 allergic patients showing positive skin reactions with the corresponding findings as reported by Alexander, Ten Cate and Franssen.

Inhalants	Personal	Alexander	Ten Cate	Franssen
1. House dust	61		93	39
2. Fungi	30		31	11
3. Grass pollen	23		26	10
4. Rye pollen	20			
5. Feathers	20	20.3	16	9
6. Cattle hair	17	8.7	14	
7. Kapok	17		19	13
8. Hay	17		53	
9. Wool	13	11.3	10	
10. Cat hair	10	9.2	18	
11. Straw mite	9			
12. Dog hair	8	8.2	11	
13. Maize pollen	5			
14. Cotton	5			
15. Horse hair	5	16.6	8	
16. Sea-weed	5			
17. Straw	4			

Food allergens	Personal	Alexander	Ten Cate
1. Chocolate	41	13.9	
2. Shrimp	39		
3. Marrowfat	34		
4. Beet	33		
5. Oatmeal	29	6.7	
6. Groats	28		
7. Pea-nut	27	3	
8. Chervil	27		
9. Spinach	27	13.3	
10. Banana	25		
11. Haricot bean	25		
12. Lean fish	25		11
13. Strawberry	24		
14. Asparagus	24		

Food allergens	Personal	Alexander	Ten Cate
15. Green peas	23	11.5	
16. Grape-fruit	20		
17. Buckwheat	19	14	
18. Lemon	19		
19. Egg	18	18.4	4
20. Onion	18		
21. Savoy cabbage	17		
22. Brown bean	17	12.9	
23. Curly kale	17		
24. Orange	16		
25. Pork	15	8.3	
26. Brussels sprouts	14		
27. Melon	13		
28. Chicken	13		
29. Swede	13	11.8	
30. Currents	12		
31. Cherry	12		
32. Potato	11	12.1	
33. Broad bean	11		
34. Horse radish	11		
35. Leek	11		
36. Turnip tops	11		
37. Purslane	11		
38. Rye	11	8.9	10
39. Parsley	10		
40. Red cabbage	10		
41. Maizena	10	5.7	
42. Cucumber	10		
43. Eel	10		
44. Cauliflower	9		
45. Endive	9		
46. Coffee	9		
47. Grape	9		
48. Herring	9		
49. Carrot	8		
50. Peach	8		
51. Pepper	8	17.5	
52. String bean	7		
53. Celery	7		
54. Chicory	7		
55. Patent flour	6		
56. Pear	6		
57. Rice	6	5.7	
58. Tomato	6	11.7	

Food allergens	Personal	Alexander	Ten Cate
59. Milk	6	14.8	5
60. Curry	6		
61. Wheat	6	20.4	12
62. Sauerkraut	6		
63. Tea	6		
64. Kidney bean	5		
65. Rhubarb	5		
66. Horse meat	5		
67. Lettuce	4		
68. Custard	4		
69. Ginger	4		
70. Nutmeg	4		
71. Beef	4	7.7	
72. Mutton	3		
73. Apple	3		
74. Tobacco	11		6
75. Barley		10.7	

a) Inhalants

1. house dust
2. fungi
3. hay
4. grass pollen
5. rye pollen
6. feathers
7. kapok

b) Food allergens

1. shrimp
2. marrowfat
3. beet
4. chocolate
5. groats
6. chervil
7. banana
8. haricot bean
9. strawberry
10. asparagus
11. spinach
12. grape-fruit
13. lemon
14. oatmeal
15. lean fish
16. green pea
17. onion
18. buckwheat flour
19. savoy cabbage
20. curly kale
21. orange

To be able to perform skin tests, a fairly large set of allergenic extracts, syringes and needles should be available.

§ 1) *The cutaneous 'screen test'*

The question is whether a single intracutaneous injection of a *mixture of all sorts of allergens* will be sufficient to determine whether or not a patient is allergic. This would enable the practitioner rapidly to decide whether complete testing is indicated in a subject, i.e. whether he is a member of the atopic group.

To begin with, it will be necessary to establish whether this method is as reliable as are the usual cutaneous and intracutaneous tests with individual allergens. This 'screen test' involves only a slight risk of systemic reactions, as higher dilutions are used.

Personal investigations

Routine testing as done in the Amsterdam Hospital for Allergic Diseases involves the use of 150 allergenic extracts, 75 of which are administered to each patient on an average.

These extracts were first arranged in 8 groups and a universal mixture was then prepared from these groups. Extreme care should be used in preparing this universal mixture. In order to avoid the risk of one extract being, for instance, one two-hundreth part of the mixture and another one-fiftieth, all solutions should be represented in an equal measure. When an intracutaneous injection of this universal extract is made into the forearm, the risk frequently will be as great as it is in cutaneous testing with a diluted allergen. This injection will only be liable to produce a larger reaction in the case of multiple sensitivity. The question was put whether it would be possible to obtain sufficient data by using the universal allergen in scratch testing. This would increase safety to a further extent. Bearing in mind that the intracutaneous test is about one hundred times more sensitive than the scratch test, it is obvious, however, that the mixture of 150 allergens as used in the scratch screen test is comparable to an intracutaneous test using a 1 : 15.000 dilution.

It is doubtful whether positive reactions can be produced by this method. When this cutaneous test was performed in 282 patients, it was found to be negative in 153 cases (54 per cent). Of these 153 patients, however, only 20 also showed a negative response to the intracutaneous test (7 per cent).

The scratch screen test was found to be of little diagnostic value in as many as 133 patients (46 per cent). The scratch screen test was negative in 11 cases (4 per cent) showing marked reactions in the group test. Curiously enough, the scratch screen test was more strongly positive than the universal intracutaneous test in two cases, the intracutaneous tests being positive in the groups. The reaction produced by one or several intracutaneous group injections was so large in 6 cases (2 per cent) in which both the scratch screen and the universal intracutaneous test were strongly positive, that testing with individual allergens was initially done again in the form of scratch testing.

These results show that scratch testing with a mixture of about 150 *allergens is of some value in establishing a tentative diagnosis and that it may be regarded as innocuous. When the test is negative, however, it should*

not be concluded that therefore the patient is not affected with atopy. Accordingly, this test should only be made as a preliminary to more extensive testing. A strongly positive test is a warning that the patient is highly hypersensitive and that there is a risk of shock.

§ 2) *The intracutaneous screen test*

This test in which the mixture of allergens is injected into the skin should be considered about a 100 times more sensitive than the cutaneous 'screen test'. This becomes apparent when the two tests are compared. To enable the use of more potent extracts as well as further differentiation, it is advisable to test all patients, including those showing a negative response to intracutaneous screen testing, with 8 groups of allergens.

These groups are made up of the following allergens:

Group I) Grass pollens (Secale cereale, Dactylis glomerata, Lolium perenne, Anthoxantum odoratum, Alopecurus pratensis, Agrostis alba, Holcus lanatus, Cynosurus cristatus); autumn flowers; summer flowers; spring flowers; maize pollen; lime pollen; bulbs (tulip, hyacinth, narcissus, gladiolus).

Group II) House dust (a mixture obtained from various houses); filling for mattresses (old kapok, wool, sea-weed, Alpine grass); furs (musk-rat, rabbit, mole, opossum, seal, pony, fox); smoke; insect powder; toilet powder.

Group III) Other inhalants: fungi; allergens on farms (duck, goose, chicken feathers); dog, cat, cattle, horse and human danders and hair; hay; straw; straw-mite; bacteria (Escherichia coli, Klebsiella pneumoniae, Micrococcus pyogenes albus, Micrococcus pyogenes aureus, haemolytic streptococci, anhaemolytic streptococci).

Group IV) Farinaceous foods and corn-chandlers' wares; rice, custard, oatmeal, groats, wheat, rye, maizena, buckwheat flour, American patent flour, yeast, peas, beans (pea in the pod, haricot bean, French bean, broad bean, green pea, brown kidney bean, white kidney bean, blue pea), macaroni, marrowfat;

Group V) Vegetables: potato, beet, swede, leek, turnip-tops, rhubarb, onion, carrot, endive, gherkin, cucumber, radish, purslane, spinach, lettuce, chicory, cauliflower, curly kale, red cabbage, savoy cabbage, Brussels sprouts, sauerkraut, asparagus, parsley.

Group VI) Dairy produce: meat and fish, cheese (4 brands, viz. Edam, Frisian, Gouda and Leyden cheese), milk, egg, meat (lamb, horse, mutton, beef, pork), duck, chicken, hare, rabbit; fish (bloater, herring, cod, haddock, plaice, pike-perch, shrimp, mussel, eel, sardine, sole, salmon).

Group VII) Fruit: strawberry, apple, grape, pear, melon, plum, currant, apricot, raspberry, cherry, grape-fruit, orange, tangerine, fig, pea-nut, almond, coco-nut, hazel-nut, walnut, tomato.

Group VIII) Luxuries: gelatine, honey, tobacco, vermicelli, beverages, chicory, cocoa, coffee, tea, nescafé, spices: pepper, Cayenne pepper, cinnamon, garlic, mustard, celery, mace, ginger, curry, nutmeg, paprika, chervil.

The intracutaneous screen test and testing with the 8 groups of extracts were compared in 675 cases, the results being recorded in tables.

The following example may serve to show how these tables were arranged.

Mrs. X is a 55-year-old woman affected with vasomotor rhinitis. The scratch screen test was +, the control test was negative and the intracutaneous screen test was found to be ++ in this patient. These results were recorded in column A of the table (cf. p. 83). The following results were obtained in the group tests: 1: ++, 2: +±, 3: +, 4: —, 5: +, 6: +±, 7: —, 8: —.

These positive reactions then were added, a + sign being recorded as 1, a ± sign as ½ and a — sign as 0. Together, this was 6½ in this case. This figure is noted in column B.

Only group I was ++, groups II and VI were +±, group V was + and group III was ±, whereas 3 groups were negative in group testing. The group tests are relatively weak in comparison with the strongly positive intracutaneous screen test. As the difference between the screen test and group testing was too marked, a ± sign was noted in column C that was assigned for this purpose, which means that the ratio between the screen test and group testing was moderately reliable.

The following classification was established on an empirical basis: when the ratio between A and B is less than 1 : 2 or greater than 1 : 10, the ratio is regarded as unreliable and a — sign is noted in column C. When the ratio ranges from 1 : 4 to 1 : 8, a + sign is noted, ± being noted in other cases. Therefore, ± was noted in column C in the case of this patient.

a) When the intracutaneous screen test was compared with testing with the 8 groups in these 675 patients, the results were found to be negative in 262 cases (39 per cent). The area of redness produced by the screen test was much smaller than that produced by group testing in 55

cases (8 per cent), in 14 cases (2 per cent) it was much larger, in 48 cases (7 per cent) it was slightly larger and in 145 cases (22 per cent) it was slightly smaller.

Of the 55 cases in which there was a marked difference between the screen and group tests, 19 (3 per cent) showed differences which were so marked that the reaction had to be considered totally unreliable in the sense that the reaction in the screen test was much too small or negative, whereas the reactions in the group tests were clearly positive.

b) The reliability of the group extracts was then studied in comparison with that of the individual allergenic extracts in 350 patients. Of these, 200 were tested with group extracts prepared in the hospital laboratory, the other 150 patients being treated with Lifa extracts. The composition of the two series of group extracts did not differ appreciably.

Both personal and Lifa group extracts were compared with the individual allergenic extracts prepared in the laboratory. The results were recorded again in a statistical table, the patient cited as an example being the same in whom the screen and group tests had been studied.

Of group I, tests with grass, rye and maize pollens as well as with spring and summer flowers were found to be clearly positive in this patient. This is well in accord with the group test in which group I was $++$. A $+$ sign was noted in the appropriate column (I) of the statistical table, which means that the group test as compared with the individual skin tests to this group is reliable in *this* case.

Of group II, house dust produced a clearly positive reaction, seaweed, wool and cottons producing $+$; therefore, $+$ was noted again in the statistical table.

Of group III, tests with fungi, feathers, dog hair and cat hair were positive. As this was $\pm$ in group testing, a proportionately too large number of extracts produced positive reactions, so that $\pm$ was noted in the case of group III.

Of group IV, white and brown kidney beans, marrowfat, buckwheat, groats and maizena produced positive reactions. This is far too much in comparison with the negative group test, so that — was recorded in group IV.

Group V was $+$ in testing with group extracts, whereas there was not a single positive reaction on testing with the individual allergenic extracts, so that this group was also recorded as negative.

Of group VI, beef, shrimp, eel and herring produced clearly positive reactions, pork and milk being $\pm$; accordingly, a $+$ sign was noted again in the statistical table.

Of group VII (negative again in group testing), strawberry and currant produced positive reactions, so that $\pm$ was noted in the statistical table.

Of group VIII, the following luxuries produced positive reactions:

tobacco, chervil, nescafé, curry. This was too much again in comparison with the group test, so that — was recorded again.

Accordingly, the statistical table was arranged as follows:

Numb. of cases	A	B	C	I	II	III	IV	V	VI	VII	VIII
675	2	6½	±	+	+	±	—	—	+	±	—

Columns I, II, etc., show the degree of reliability of the group extracts in comparison with the individual allergenic extracts of the group. Moreover, this provides a simple method by which to determine whether the extracts of the group are sufficiently reliable.

The statistical table took on the form as shown in Table XV.

TABLE XV

Case	A	B	C	I	II	III	IV	V	VI	VII	VIII
1	½	8	—	+	+	+	+	+	+	±	+
2	½	5½	—	+	—	+	+	±	+	+	+
3	½	8½	—	+	+	±	+	+	+	+	+
4	2	12	+	+	+	+	+	+	+	+	+
5	2½	8½	±	+	+	+	+	+	—	+	+
6	2	11	+	±	+	±	+	+	±	±	+
7	2	7½	±	+	—	+	+	+	+	+	±
8	—	1	+	+	+	±	±	+	+	+	+
9	1½	6	+	+	+	+	+	+	+	—	+
10	½	5½	—	±	+	+	+	+	±	±	+
11	½	5	±	±	—	+	+	+	—	±	+
12	½	2	+	+	+	+	+	+	+	—	+
13	1½	9	+	+	+	+	—	+	—	+	+
14	1	5	+	+	—	+	—	+	±	—	+
15	1	6½	+	+	+	+	+	—	±	+	±
16	½	4½	±	+	+	+	—	±	+	—	+
17	1	4½	+	±	±	+	±	+	+	±	+

Adding all + and ± signs in columns I to VIII inclusive provides a survey of the reliability of the group extracts in comparison with the individual allergenic extracts. The number of + signs, etc., for group I were found to be 172 in 200 personal controls (the maximum would have been 200), so that the reliability of group I was rated at 86 per cent.

These calculations showed that there was only a slight difference in favour of the personal extracts between the reactions to Lifa extracts and those to the personal group extracts.

The mean percentages for the personal group extracts were as follows:

I: 86 per cent	IV: 77 per cent	VI: 76 per cent
II: 84.5 per cent	V: 59 per cent	VII: 60.25 per cent
III: 84 per cent		VIII: 76 per cent

The mean percentages for the Lifa group extracts were:

I: 70.5 per cent	IV: 67.34 per cent	VI: 64 per cent
II: 78.67 per cent	V: 42.67 per cent	VII: 57.5 per cent
III: 68 per cent		VIII: 66.34 per cent

As regards inhalants, (I - II and III), the mean percentage for the personal group and allergenic extracts therefore was 85 per cent, that for the Lifa extracts being 73 per cent, and those for the extracts of foods being 68 per cent and 60 per cent respectively (IV to VIII included).

These studies also show the reliability of the tests with inhalants to be considerably superior to that of the tests with food allergens, the variations of the latter being most marked in the vegetable and fruit groups.

1) The question arises whether this difference might possibly be due to the presence of *seed* in the material from which these extracts were prepared.

Therefore, extracts were prepared from various seeds and compared with the individual allergen in question and with the group extract in which this allergen was included. Tests were done with the pips or seeds of pears, apples, currants, tomatoes, strawberries, lemons, oranges, tangerines, grape-fruit, bananas, grapes, cucumbers, gherkins, almonds and figs; in addition, tests were made with pea-nuts and walnut as well as with soft and hard roes of fish and shrimps as related propagative allergens. A mixture of these 20 extracts was prepared again and diluted to 1 : 50 (so that each of these propagative agents was present in a 1 : 1.000 dilution). Another mixture was diluted to 1 : 10 (= 1 : 200) and a third to 1 : 5 (= 1 : 100).

Of 30 patients in whom the above tests with fruit, fish and shrimp were more or less positive and who had been so selected that they showed positive responses to 1 : 1000.000 histamine, 6 showed a positive response to the 1 : 50 dilution, 2 subjects reacting with ++ and one with ++++. Of the 24 others, 2 reacted to the 1 : 10 dilution with ++, 3 reacted with +, and the other 19 were tested once again with the 1 : 5 dilution. Of these, 8 showed a positive response, one reacting with ++, one with +++ and one with ++++. Of the remaining 11 patients, two more showed a positive response to the undiluted mixture. The propagative extracts were also used in simple scratch tests on the 19

patients who had shown the least intensive reactions to screen testing. Of these tests, 62 per cent were positive, one reaction being characterized by marked pseudopods!

Van Dishoeck [33] compared extracts of grass-seed with pollen extracts. Reactions to the former were much weaker. Pollen, however, also is a propagative allergen.

Conclusion: These tests show that seed undoubtedly is a potent allergen.

2) Group allergenic extracts, from which a number of seed allergens had been removed, were subsequently prepared by Lifa and compared with personal allergenic extracts in 160 cases, using the methods as described under *b* (cf. p. 82). Personal group extracts devoid of all the above propagative substances were also compared with the corresponding allergenic extracts in 165 cases. The mean variation percentages were not found to show any significant improvement in the two groups, the percentages for the Lifa group extracts IV to VIII inclusive showing an improvement from: 76 per cent, 43 per cent, 64 per cent, 58 per cent and 66 per cent to 66 per cent, 56 per cent, 70 per cent, 76 per cent and 74 per cent, i.e. from a mean percentage of 60 to 68, and those for the personal group extracts improving from: 77 per cent, 59 per cent, 67 per cent, 60 per cent and 76 per cent to 77 per cent, 73 per cent, 89 per cent, 84 per cent and 84 per cent, i.e. from a mean percentage of 68 to 81.

The difference does seem to be significant at a first glance, but the percentage is found to be much higher in group VIII, the composition of which naturally underwent only few alterations, whereas there actually were no changes at all in group IV, from which a relatively large number of allergens were removed, although variations were much more likely to occur in this case. Accordingly, there must be other factors affecting the skin reactions (cf. Chapter IV).

§ 3) *Is it advisable to use fresh personal extracts in preference to commercial extracts?*

The skin reactions produced by commercial extracts (Lifa, Bencard, Barfod and Antibody Products Ltd.) were compared in a number of cases (the intensity of the skin reactions was stated in figures in the table).

To begin with, a house allergenic extract was tested in 21 patients [see Table XVI].

TABLE XVI

House dust extract

Case	Personal	Lifa	Bencard	Barfod	AP Ltd.
1	1	½	½	—	½
2	1	—	—	1	½
3	2	1	1½	1	1½
4	3½	2½	3½	3	3
5	4½	4½	4	4½	5
6	2	½	½	½	1½
7	3	2	2½	2½	2½
8	2½	2	½	1½	1½
9	1½	1	1	2	2
10	3	2	3	3	2½
11	3	3	2½	2	2
12	2	1½	½	½	½
13	½	1	—	—	½
14	2½	2	2	2	1½
15	2½	2½	2½	2	1½
16	2	1	1	2	1½
17	½	—	½	½	1
18	½	½	1	½	½
19	4	3½	3½	3	3½
20	2	1½	½	1	1
21	1½	1	1½	1	2
Total	45	33½	32½	33½	36

An extract of chocolate was tested in 6 cases:

Case	Personal	Lifa	Bencard	Barfod	AP Ltd.
1	2	1½	1½	1½	2
2	1½	2½	2	2	1½
3	1½	2	2½	2	2
4	2	2	2½	2	3½
5	2½	2	2	1	2
6	2½	1½	1	1	½
Total	12	11½	11½	9½	11½

In addition, the following extracts were tested:

	Personal	Lifa	Bencard	Barfod
Buckwheat	1	0	0.5	
Chocolate	1	0.5	0	
Chocolate	2	0.5	0.5	1
Fish	1	0	0	
Fungi	2	0	0.5	0
Fungi	0.5	0.5	0	
Fungi	1	2	1	1
Grass pollen	0.5	0.5	1	
	9	4	3.5	2

In 35 extracts, the ratios of the personal extracts to the Lifa and Bencard extracts were: 66 : 49 : 47.5 respectively or, converted to 100 : 100 : 74 : 72 per cent respectively, the ratios in the 27 cases tested with 5 types of extract being: 57 : 45 : 44 : 43 : 47.5 or, expressed as percentages: 100 : 79 : 77 : 75 : 83 per cent.

Conclusion: The use of freshly prepared extracts is to be preferred in experimental studies; commercial extracts, which undoubtedly yield fairly satisfactory results, may be used in clinical cases, though there may be some difficulty in the interpretation of weakly positive reactions. So far, there were not found to be any significant differences between the various commercial extracts.

§ 4) *What happens when allergens are arranged in groups?*

One possibility is potentiation, or a subliminal sensitivity might become positive under the influence of one or several other subliminal sensitivities.

Moreover, it should be borne in mind that a large number of irritating substances are introduced into the skin along with the active allergen; the combined action of these substances might be liable to cause non-specific irritation.

Personal investigations

1) To study this matter, an extract of fungi was prepared, which was so potent that the reactions produced in patients sensitive to this extract and to pollen extract were similar to those produced by a mixture of 100 units of grass pollen plus fungi [Table XVII]:

TABLE XVII

	First month		Second month		Third month	
Case	Pollen+fungi	Fungi	Pollen+fungi	Fungi	Pollen+fungi	Fungi
1	++±	+	+	++	+±	+±
2	±	+	—	+	—	+
3	—	+	±	+	+	+
4	—	+	+	±	+	+
5	++	+	+	±	+	+
6	+	+	+	—	+	+
7	+	±	+	+±	+	+±
8	±	±	+	±	+	—
	7½	7	6½	7	7½	8

A few ampoules of the two mixtures were stored in the refrigerator. At one-month intervals, an ampoule of each extract was taken from the refrigerator, the extracts being injected into a number of subjects allergic to pollen and fungi the very same day. The ampoules were destroyed after use. These tests showed that pollen and fungus allergens may be combined without reducing or increasing the action of the mixture during the course of a few months.

2) The mixture of extracts of inhalants, viz., grass and rye pollens, phlox and lime blossom, with fish extracts showed that these substances also do not interact.

3) The extracts of a number of inhalants producing positive reactions in 15 patients were combined (desensitizing solution).

Intracutaneous tests with these inhalants as well as tests with the mixture were then performed twice at 6-week intervals in these cases. When the two reactions were compared with each other and with the results of the test made about 6 weeks previously, no appreciable difference was observed. This shows that the combination of various allergenic extracts again did not have any disturbing effect.

The last results have been listed in Table XVIII.

The results obtained on the first day in the first series of intracutaneous tests have been recorded in figures in column one of this table, those obtained on the first day in the second series of tests performed about 6

weeks later having been listed in column two. The desensitizing solutions were prepared as follows: 1 ml. of 1 per cent of every extract producing positive reactions was made up to 10 ml. with 5 per cent house allergenic extracts and of 5 per cent extracts of fungi āā. When the second series of tests were performed, two other injections were made in addition to injections of the individual allergenic extracts, viz., injections of the desensitizing solution which now was 6 weeks old and had *not* been stored in the refrigerator (column D_1) as well as of a *fresh* desensitizing solution made up of the same ingredients (column D_2).[1] The total number of points scored in the first series of skin tests was 197 in 15 patients, that scored in the second series being 208; a negligible difference of 5.6 per cent.

The figures recorded in the columns listing the results of testing with the desensitizing solutions produced totals of 33.5 and 38.5 respectively, a difference of 15 per cent.

Accordingly, the potency of the mixture had declined rather than been potentiated after about 6 weeks.

Conclusion: The arrangement of allergenic extracts in groups is a rational method, designed not only to supply a rapid answer to the question 'allergic or non-allergic?', but also to enable the practitioner to decide whether the principal allergens are among particular foods (dairy produce, luxuries) or among particular groups of inhalants.

Subsequent grouping in so-called screen tests will increase saftey and intracutaneous screen testing provides a fairly reliable answer to the question whether or not a patient is allergic. Eleven syringes and needles (including one for testing to histamine and another for control tests) as well as a number of vials containing extracts of groups of allergens, a vial of 1 : 10.000 histamine and a control vial will enable investigations not involving too high a cost. Commercial allergenic extracts may be used in testing.

Other standards should be adopted by specialists, concerned with the study of allergy; in this case, it is advisable to start with group testing as a matter of security and then to perform more differentiated tests, preferably using freshly prepared personal allergenic extracts.

[1]) In columns D_1 and D_2, the delution of the desensitizing fluids used in administration is stated at the top.

TABLE XVIII

I.	I	II	D_1	D_2
House all. 1 %	2½	3	1/10	1/10
Fungi 1 %	½	1½		
Wool 1 %	1½	1		
Mites 1 %	2½	2		
Rye poll. 500 U	1	1½		
Cotton 1 %	1½	½	3½	3
	9½	9½		

II.				
House all. 1 %	4	4	1/10	1/10
Fungi 1 %	2	2		
Grass poll. 50 U	1½	1		
Rye poll. 100 U	1	2		
Tobacco 1 %	1	½	3½	2½
	9½	9½		

III.				
Fungi 1 %	4	4	1/100	1/100
Grass poll. 100 U	2	3		
Feathers 1 %	1	2		
Cotton 1 %	1	1		
Hyacinth 1 %	3	3		
Phlox 1 %	1½	½		
(Mixed) fish 1%	2½	2½	2½	3½
	15	16		

IV.				
House all. 1 %	2	1	1/10	1/10
Fungi 1 %	2	2		
Tobacco 1 %	1	½		
Horse hair 1 %	3	4		
Feathers 1 %	3	3		
Mites 1 %	3	2	2	2½
	14	12½		

V.	I	II	D_1	D_2
House all. 1 %	1	3	1/100	1/100
Fungi 1 %	2	3½		
Tobacco 1 %	3	4		
Dog dander 1%	3	3		
Feathers 1 %	2½	1½		
Old Kapok 1 %	2½	1		
Wool 1 %	2½	1		
Cotton 1 %	2	1		
Grass pollen 100 U	2	1½		
Rye poll. 100 U	3	3	2½	2
	23½	22½		

VI.				
Feathers 1 %	2½	1	1/10	1/10
Old Kapok 1%	2	2		
Wool 1 %	2	1½		
Cotton 1 %	1	1		
House all. 1 %	1	2½		
Fungi 1 %	2	1½		
Grass pollen 100 U	2	1½		
Rye poll. 100 U	2½	2½	1½	2
	15	13½		

VII.				
House all. 1 %	2½	3½	1/10	1/10
Fungi 1 %	2	1		
Hay 1 %	1½	2		
Straw 1 %	1½	4		
Feathers 1 %	2½	2		
Old Kapok 1%	1½	2		
Bacteria 6.M.U.	3	3½	3	1½
	14½	18		

VIII.	I	II	D_1	D_2
House all. 1 %	2	4	1/1	1/1
Fungi 1 %	½	1		
Feathers 1 %	1½	1		
Wool 1 %	½	½		
Grass pollen 100 U	1½	1½		
Rye poll. 100 U	½	½	2	3
	6½	8½		

IX.				
House all. 1 %	2½	4	1/100	1/100
Fungi 1 %	1½	1		
Feathers 1 %	3½	2		
Old kapok 1 %	2	1		
Mites 1 %	3	3½	2	3
	12½	11½		

X.				
House all. 1 %	1½	4	1/10	1/10
Fungi 1 %	½	1		
Wool 1 %	1½	2		
Old kapok 1 %	2	1	2	2
	5½	8		

XI.				
House all. 1 %	2½	3	1/10	1/10
Fungi 1 %	3½	4		
Old kapok 1 %	2½	1½		
Wool 1 %	2	½		
Cotton 1 %	1½	1½		
Grasspollen 100 U	1½	2		
Rye poll. 100 U	1	2½	2	3
	14½	15		

XII.	I	II	D_1	D_2
House all. 1 %	3	3	1/100	1/100
Fungi 1 %	1½	1		
Old kapok 1 %	1½	1		
Wool 1 %	1½	2		
Feathers 1 %	½	1		
Mites 1 %	2½	1		
Grass pollen 100 U	1½	3		
Rye poll. 100 U	3	4	1½	2½
	15	16		

XIII.				
House all. 1 %	3	3½	1/100	1/100
Fungi 1 %	3	3½		
Grass pollen 250 U	1	1½		
Rye poll. 250 U	1	1½		
Tulip 1 %	1½	1½		
Hyacinth 1 %	3	1½		
Gladiolus 1 %	3	1½		
Narcissus bulbs 1 %	3	2½	1½	2
	18½	17		

XIV.				
House all. 1 %	2	3	1/10	1/10
Fungi 1 %	1½	3		
Dog 1 %	2½	3		
Feathers 1 %	2	1		
Old kapok 1 %	2	2		
Wool 1 %	2	3		
Grass pollen 100 U	2	3		
Rye poll. 100 U	2	3	2	2½
	16	21		

XV.				
House all. 1 %	2	3½	1/10	1/10
Fungi 1 %	1½	3		
Grass pollen 100 U	2	2		
Rye poll. 100 U	2	1	2	3½
	7½	9½		

SUMMARY

Although the technique used in skin testing is imperfect and the results are difficult to interpret, it frequently is the only method by which to determine whether or not a patient is allergic. Moreover, it provides the only basis for treatment by elimination and specific desensitization.

The difference in size and the variability of the skin reactions will depend on the nature of the allergen, the potency of the allergenic extract, the site of injection, the circulation within the vessels of the skin, the age, character and condition of the patient, the foods ingested shortly prior to skin testing and whether or not he has been treated with certain drugs. False reactions may result from errors of technique, from technical errors committed in preparing the extract or from irritation caused by the preservative. There also may be a period of refractoriness. The gastric and intestinal juices may reduce the potency of an allergen. In addition, latent allergy may be present.

To begin with, the two types of allergic reaction were reviewed: immediate and so-called delayed reactions.

Immediate, anaphylactic, atopic reactions will become positive within from 10 to 30 minutes, *delayed*, bacterial, tuberculin, eczematous reactions becoming positive within from 2 to 48 hours. The immediate reactions include:

1) the Arthur phenomenon,
2) anaphylactic shock,
3) so-called atopic reactions such as serum disease, asthma, pollinosis, urticaria, angioneurotic oedema, periarteritis nodosa (possibly, also rheumatic fever, glomerulonephritis and rheumatoid arthritis).

The delayed reactions include:

1) reactions to parasites,
2) contacts with chemicals, primrose, etc.

Immediate skin reactions are produced in scratch, prick, 'bore' and intracutaneous testing.

Delayed skin reactions occur in the scratch test(von Pirquet's test),intracutaneous testing and patch testing.

The technique of skin testing in allergy was studied.

The techniques of the scratch, 'bore', prick and modified prick tests were reviewed and the results obtained in tests performed in 50 cases compared. These studies showed that scratch testing with the vaccino-style is the most suitable method of epidermal testing.

The technique of patch testing and the problems relating to this matter were discussed. Performing patch tests was found to be a typical specialists' job.

Subsequently, the problem of freeing the syringes and needles from allergens was studied. Ten times' cleansing with Coca's solution did not result in the disappearance of all allergens from the syringes and needles, and all skin tests were still found to be more or less positive up to an average of 45 per cent of the initial values. Even when it had been boiled for 30 minutes, an average proportion of 25 per cent of the allergenic extract was left in the syringes. This proportion showed a marked decrease following preliminary rough cleansing of the instruments.

To ensure thorough cleansing, 3 per cent H_2O_2 should first be passed through the needles and syringes to bring about the disintegration of remnants of blood, if any, followed by cleansing with physiological saline or 0.5 per cent phenol. A mandrin should be passed through the needles as a preliminary measure in every case. This is followed by mechanical cleansing, the syringes being taken to pieces and brushed with desogen, lodaline or typol. The syringes and needles are then boiled in a pan for 5 minutes; the instruments are taken out, allowed to drain and boiled again in another pan for 30 minutes, the needles being suspended in a gauze net. Care should be taken to ensure that no air-bubbles are left in the syringe. The pH of the fluid is not of any importance in freeing the syringes from allergens.

Prior to testing, the skin should be thoroughly cleansed with soap and water, then treated with other agents, preferably ether, and finally disinfected, e.g. with zephiran, etc. Owing to its rapid evaporation, the ether will not produce any local anaesthesia persisting beyond 2 minutes.

Prolonged differences of temperature will affect the skin reactions. Exposure to low temperatures is sufficient to reduce the degree of reactivity for 20 minutes; exposure to high temperatures resulting in an increase in size of the skin reaction for 30 minutes. When patients come to the surgery in winter, they should remain in a heated room for thirty minutes prior to injection of the allergenic extracts.

The test site is an important factor. Though the skin of the back shows the most marked reactions, the forearm or thigh are to be preferred in daily practice. These two sites offer the great advantage that the extremity may be ligated in the event of imminent shock.

Treatment with antihistaminics a few hours before skin testing is not advisable. Subcutaneous injection of 0.5 ml. of 1 : 1.000 adrenaline will undoubtedly have an inhibitory effect on the reactivity of the skin, which effect is likely to persist for 60 minutes after administration.

From a clinical point of view it is of importance to know whether all allergenic extracts can be sterilized and what their reactions will be to prolonged boiling and boiling at high temperatures. All allergens are found to be sterilizable without any marked decline in potency, continued boiling for 4 hours reducing the action to about one-tenth of the initial action; the action then continues at a constant level. The potency only

shows a marked decline following 30 minutes' boiling at 180° and the extracts are affected by a temperature of 300°.

Sterilization of the allergens alone is not an effective method in general practice, as the liquid will immediately have to be transferred to ampoules in that case, which ampoules have to be destroyed after they have been used once. Chemical sterilization with Coca's solution or 0.5 per cent phenol is the most effective measure. Preliminary sterilization of the extracts is advisable.

Mechanical sterilization results in a greater or lesser decline in potency of the extracts; Jena glass filters (bacteria-tight filter C 5) may be used for this purpose. Sterilization by passing the extracts through Seitz filters is followed by a marked decrease in potency. Extraction of the allergens by alcohol occasionally results in a loss of potency of about 50 per cent.

The optimum effect of allergenic extracts varies with the pH level. The extract is most potent at pH 7.5, the allergenic action decreasing by about 10 per cent at pH 6.5 and 8.5. When the pH is below 6.0 or above 9.0, non-specific reactions will result.

It is difficult to maintain the pH of allergenic extracts at a constant level unless they are kept in air-tight small ampoules. Correcting a pH that has decreased, which is liable to occur in the case of extracts of food allergens, has the drawback that it results in dilution of the extract.

A problem of vital importance in general practice is that of keeping allergenic extracts free from bacteria. When an extract is employed at regular intervals (for instance, 3 times a week), bacteria will be found to be present within 3 months when the extract is stored in the refrigerator and within 2 months when it is stored at room temperature; this will occur despite so-called autosterility. Accordingly, allergenic extracts should be replaced by fresh extracts every 2 months and the quantity of desensitizing fluid should not exceed the amount required for desensitization purposes for 2 months (bottles containing about 10 ml. of fluid). The above shows that skin testing by practitioners who only carry out this investigation occasionally involves a great number of difficulties.

When it is necessary to make sure whether a skin test is positive in doubtful cases, the Prausnitz-Küstner test may be performed. There are drawbacks to this method, however, as it is a passive transfer test in which serum of a specifically allergic subject is injected into the skin of a normal individual. Therefore, tests for syphilis should be done prior to performing the Prausnitz-Küstner test and efforts should be made to rule out the presence of virus disease. To ensure scientific testing, it is essential that a maximum amount of reagins should be present in a circumscribed area of the skin. A compression ring was made for this purpose; this ring has a notch on one side and a groove slanting inwards was ground in this notch to ensure that the needle used in injections will

always have to be inserted into the skin in the axis of the circle. Three small concavities in the side of the ring result in the appearance of three small areas following compression. These areas should be marked so that they will be identifiable for as long as week, thereby indicating exactly where and how the second injection of the allergenic extract should be made.

As the making of a large number of intracutaneous injections is unpleasant as well as dangerous to the patient, as it takes up much of the physician's time and also involves the use of a large amount of materials, the question was put as to whether there was any point in confining the tests to intracutaneous injections of those substances which produce the greatest number of positive reactions.

To ensure a reasonable degree of accuracy, at least 7 extracts of inhalants and 21 extracts of foods should be available. Even then, however, a fairly gross error will be committed in addition to the technical errors naturally attaching to intracutaneous testing.

Therefore, efforts were made to develop a simpler method by which to decide whether or not a particular patient is allergic. It is advisable to start by making an injection of a solution in which all allergens are present in equal proportions. This will enable an initial screening of the patient. To prevent shock, scratch testing may be substituted for the injection. This will produce a degree of sensitivity equivalent to a dilution of about 1 : 15.000. The intracutaneous screen test was found to provide reliable data on the presence of allergy in about 60 per cent of the cases, the test being entirely unreliable in 3 per cent of the cases.

In the event of a positive screen test, the patient should be tested to group extracts. The allergenic extracts were arranged in 8 groups. Group I consisted of pollens and pollen grains, group II included all house inhalants, group III included all allergens in the outdoor atmosphere, group IV consisted of flour and corn-chandler's wares, group V of vegetables, group VI of meat, fish and dairy produce, group VII of fruit, and group VIII of luxuries.

When comparative testing was done with these group extracts and personal extracts of individual allergens, satisfactory results were obtained in an average proportion of $72^1/_3$ per cent of the cases using Lifa extracts of inhalants and 85 per cent of the cases using personal extracts. This shows that commercial extracts may be used succesfully in general practice. Comparative studies did not reveal any significant difference between the extracts prepared by various factories. In scientific testing, however, personal freshly prepared extracts are to be preferred to commercial extracts.

The results obtained in tests with group extracts of food allergens are less reliable; especially the results obtained in testing with group extracts of vegetables and fruit are below the useful average. The variable results

obtained in testing with these allergens possibly is due in part to the fact that certain so-called simple allergenic extracts none the less consist of various substances which may produce different reactions; thus it may happen that patients will show intensive reactions to so-called propagative organisms (soft and hard roes of fish, fruit seeds and vegetable-seeds). Moreover, the season may affect the results obtained in testing. Tests with inhalants are again found to produce the most reliable results in these cases. It is advisable to perform skin tests outside the season in which the patient shows allergic manifestations, wherever this is possible.

There was no evidence of potentiation or disintegration of the constituents of group allergenic extracts, which suggests that testing with from 6 to 8 groups will enable the physician to decide with a fair degree of accuracy whether or not a patient is allergic. Only 11 syringes are required in this investigation.

An evaluation of the reactions based on the size of the latter is of great importance in comparing the results obtained in testing. Opinions on the most effective method of evaluation, however, vary markedly. Personal investigations and comparison of the results of an international inquiry, to which 30 physicians concerned with the study of allergic conditions replied, showed that the following method is to be preferred:

In making an intracutaneous test, 0.02 ml. of allergenic extract should be injected, 0.02 ml. of preserving fluid as well as 0.02 ml. of 1 : 10.000 histamine being injected as a control test. The control test should be negative; in testing with a 1 : 10.000 dilution of histamine, both the size of the wheal and that of the areola were adopted as standards in evaluating the reactions. As tests with 1 : 1.000.000 histamine usually are just positive, 1 : 10.000 histamine is designated as +++. Pseudopods were found to merit an additional ++ in evaluating skin reactions. In conclusion, it is pointed out that all skin reactions should be read in the same place and in the same lighting.

To enable comparisons on an international scale, factories manufacturing allergenic extracts should briefly state the method used in preparing the extracts on their labels, in addition to the date of extraction and the percentage or number of units.

CONCLUSIONS

1) Parmi les cutiréactions il faut préférer le scratchtest plutôt que l'expérience par piqûre ou forage, parce que le scratchtest est de loin le moins désagréable pour le patient.

2) Il faut juger les cutiréactions par rapport à la réaction au moyen d'histamine 1/10.000 et à une réaction de contrôle au moyen du liquide extrait. A la réaction au moyen d'histamine 1/10.000 il faut décerner +++, pour les expériences intracutanées il faut injecter 0.02 cc. Il faut honorer les pseudopodies d'un supplément de ++.

3) Pour le nettoyage des seringues injectrices et des aiguilles le nettoyage mécanique est très important. Avant de le stériliser, il faut d'abord faire bouillir le matériel infecté de matières allergiques pendant 5 minutes; après égouttement il faut encore le stériliser dans un autre stérilisateur. Il ne faut pas faire bouillir dans une position horizontale.
Il faut strictement tenir séparés le matériel pour l'examination et celui servant à des fins thérapeutiques.

4) Pour effectuer les expériences cutanées il faut nettoyer la peau avec de l'éther. Après 2 minutes l'éther n'a plus aucune influence sur le volume des cutiréactions. L'adrénaline n'a plus d'influence sur le résultat après 60 minutes; l'effet de Théphorin est très variable. Il faut donc déconseiller l'emploi de Théphorin, de même que l'emploi d'autres antihistaminiques peu de temps avant qu'on effectue une expérience cutanée.

5) Il s'avère que tous les allergènes sont thermostabiles, qu'une ébulition prolongée ne réduit la puissance allergène qu'après 2 heures, que cela dure 30 minutes à une température de 180° et que les allergènes ne supportent pas les températures très élevées (300°).
La stérilisation chimique au moyen du liquide de Coca ou de $\frac{1}{2}$ % de Phénol n'influence que très peu la cutiréaction; à la filtration on constate bien une perte d'allergène; à la filtration à verre Jena la perte est la plus petite; à la filtration Seitz la perte est considérable.

6) En général la stérilisation chimique ne freine que pendant 10 semaines en moyenne le développement de bactéries dans les extraits allergènes. Il faut surtout tenir compte avec cela à l'emploi

de liquides de désensibilisation. Le pH du liquide extrait est de peu d'importance, tant en ce qui concerne le développement de bactéries que la puissance des extraits.

7) Il est important de rationaliser l'expérimentation des allergènes par un groupement d'allergènes. La continuation du groupement jusqu'à un mélange de tout, mélange dit 'screen' est surtout d' importance pour prévenir le risque de shock à l'expérimentation fréquente de routine. Combinées avec des groupes d'extraits allergènes on obtient avec 11 injections une opinion assez sûre pour savoir si un patient est du groupe atopique.

8) Il se trouve que les allergènes d'inhalation sont les plus sûres et que parmi les produits alimentaires ce sont surtout les légumes et les fruits qui nous abandonnent plus d'une fois. Outre les matières dites propagatives d'autres facteurs inconnus jouent un rôle à côté des influences quotidiennes et saisonnières.

9) Pour l'expérimentation il faut se servir d'extraits de propre fabrication; pour les examens de routine on peut très bien se servir des extraits commerciaux.

10) La réaction de Prausnitz-Küstner est surtout importante pour des recherches quantitatives pourvu qu'on se serve à cet effet d'un compresseur.

SCHLUSSFOLGERUNG

1) Von den kutanen Hautreaktionen ist das Skarifikationsverfahren der Stich-und Bohrprobe vorzuziehen, weil der erstere weitaus am wenigsten unangenehm für den Patienten ist.

2) Die Hautreaktionen müssen beurteilt werden gegenüber einer Reaktion mit Histamin 1/10.000 und einer Kontrollreaktion mit der Extraktflüssigkeit.
Die Histaminreaktion 1/10.000 muss mit +++ bewertet werden. Bei den intrakutanen Hautproben muss 0.02 cc eingespritzt werden. Pseudopodien werden mit ++ extra bewertet.

3) Beim Reinigen von Spritzen und Nadeln ist die mechanische Reinigung sehr wichtig. Vor der Sterilisation muss das mit Allergenen verunreinigte Material zuerst 5 Minuten lang gekocht werden, wonach nach dem Abtropfen die Sterilisation in einer anderen Pfanne folgen muss. Das Kochen darf nicht in horizontaler Lage vorgenommen werden.

4) Vor der Ausführung von Hautproben muss die Haut mit Aether gereinigt werden. Aether hat nach 2 Minuten keinen Einfluss mehr auf die Grösse der Hautreaktionen. Adrenalin beeinflusst das Resultat nach 60 Minuten nicht mehr. Die Wirkung von Thephorin ist sehr wechselnd. Der Gebrauch desselben, und das gilt auch für andere Antihistaminica, muss abgeraten werden, wenn kurz danach eine Hautprobe ausgeführt werden soll.

5) Es hat sich gezeigt, dass alle Allergene thermostabil sind, dass langes Kochen die Allergenpotenz erst nach 2 Stunden vermindert, dass dies bei 180° eine halbe Stunde dauert und dass Allergene sehr hohe Temperaturen (300°) nicht vertragen.
Chemische Sterilisation mit Coca's Flüssigkeit oder $\frac{1}{2}\%$ Phenol hat einen sehr geringen Einfluss auf die Hautreaktion. Beim Filtrieren tritt wohl Allergenverlust auf, am wenigsten bei Jenaglas filtrieren, jedoch bei Seitzfiltrieren ziemlich beträchtlich.

6) Chemische Sterilisation hemmt im allgemeinen nur 10 Wochen lang das Bakterienwachstum in den Allergenextrakten. Hiermit

muss man vor allem bei den Desensibilisierungsflüssigkeiten rechnen. Der pH der Extraktflüssigkeit ist nicht wichtig sowohl was das Bakterienwachstum, als was die Potenz der Extrakte betrifft.

7) Rationalisierung der Allergenuntersuchung durch Gruppierung von Allergenen ist wichtig. Das Durchgruppieren zu einer Mischung aller Allergene, ein sogenannter „screen" ist vor allem wichtig zur Vorbeugung des Risikos von Shock bei gehäufter Routinen-untersuchung; zusammen mit Gruppen von Allergenextrakten erhält man mit 11 Injektionen einen ziemlich sicheren Einblick, ob ein Patient zu der atopischen Gruppe gehört.

8) Es zeigt sich, dass die Inhalationsallergene am zuverlässigsten sind, und dass unter den Nahrungsmitteln vor allem die Gemüse und Obstsorten uns manchmal im Stich lassen. Ausser sogenannten „propagativen Stoffen" speilen neben Tag- und Säsoneinflüssen noch andere unbekannte Faktoren eine Rolle.

9) Für experimentelle Untersuchungen muss man von selbstgemachten frischen Extrakten Gebrauch machen, für Routinenuntersuchungen sind die im Handel erhältlichen Extrakte gut brauchbar.

10) Die Prausnitz-Küstner-Reaktion ist vor allem wichtig für die quantitative Untersuchung, wenn man dabei von einem Kompressor gebrauch macht.

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STELLINGEN

STELLINGEN

I

De meeste allergenen zijn geen eiwitten, maar verkrijgen hun antigene eigenschappen door koppeling aan serumeiwitten.

II

Sterilisatie van spuiten en naalden behoort niet in horizontale stand te geschieden.

N.T.v.G. 103 I-II-1959, 573.

III

Men behoort huidproeven met allergenen niet te doen wanneer de patiënt in een acute allergische phase verkeert.

Storm van Leeuwen, W. en van Niekerk, J., *Zschr. f. Imm. Forsch.* Vol 62, No. 5-6, 1939, p. 405-410.
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IV

De suggestie om in militaire dienst iedereen met keelinfectie door haemolytische streptococcen met antibiotica te behandelen is gevaarlijk.

Ned. Mil. Geneesk. Tijdschr. Jrg. XII-3-59 p. 57-66; 6-59 p. 159-164; 8-59, p. 229-230: 9-59 p. 263-273.

V

De constitutionele factor is van secundair belang bij het ontstaan van longkanker.

Korteweg, Dr. R. Huisarts, roken en de preventie van longkanker. *Overdruk Verslagen en Med. Volksgezh.* 8-9 (1958), p. 34.

VI

Men dient niet over te gaan tot bestraling, of behandeling met cortisone van eczemen, vóór ook de mogelijkheid van een allergische aetiologie is onderzocht.

VII

Bij volwassen cataractpatiënten onder het 60e jaar kan men een intracapsulaire lensextractie verrichten met behulp van α chymotrypsine.

Survey of Ophthalmology, vol 3-4-VIII-1958, p. 299-303.
La Revue Chibret, no. 32 p. 13-32.

VIII

Een gunstige calciumwerking bij allergische ziekten berust op het minder doorlaatbaar worden van de gesensibiliseerde endotheelcellen der bloedcapillairen.

Acta Allergologica 1958 - suppl. V. 259-280.

IX

Onderzoek naar het aeroplankton voor zover betreft de fungi buitenshuis, binnenshuis en in bedrijven heeft alleen zin, wanneer een methode wordt gekozen, die identificatie tot op de species mogelijk maakt.

P. J. van der Werff, *Mould Fungi and Bronchial Asthma*. Vol I 1958, p. 25, 26 en 36.
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X

In de komende wereld zal uiteindelijk blijken, dat de zieke mens het meest gebaat is door hulp van de vrije artsen.

Bull. W. M. A. April 1949 no. 1.
Bull. W. M. A. Juli 1950 no. 3.
Het Ziekenfonds. April 1956; no. 4, pag. 17.

CURRICULUM VITAE

Na de Rijks Hogere Burgerschool te Middelburg te hebben doorlopen, liet promovendus zich, door zijn vader mede gestimuleerd, in 1928 inschrijven aan de Rijks Universiteit van Utrecht voor de studie in de Geneeskunde.

Toen hem de gelegenheid werd geboden de lange wachttijd tussen het semi-artsexamen en het begin der co-assistentschappen, in Frankrijk productief te maken, bezocht hij in 1936 gedurende geruime tijd enige ziekenhuizen in Parijs. Vooral tijdens de studie in het Hôpital Broussais werd aan de cardiologische afdeling de belangstelling voor de Inwendige Geneeskunde gewekt.

Na het afleggen van het Artsexamen in 1937 volgden enige jaren assistentschap aan de afdeling Inwendige Ziekten van het Gemeente Ziekenhuis in Den Haag, gevolgd door een jaar studie aan dezelfde afdeling van de Rijks Universiteit te Utrecht.

Na zijn inschrijving in het specialisten-register voor Inwendige Ziekten met aantekening Röntgenologie vestigde hij zich op Schouwen Duiveland en werd verbonden aan het ziekenhuis aldaar en van 1947 af aan die van Coevorden en Hardenberg. In 1953 verbond hij zich aan de Amsterdamsche Kliniek voor Allergische Ziekten en werd hij tevens aan de Leidse Universiteitskliniek op de afdeling voor Keel-, Neus- en Oorheelkunde in de gelegenheid gesteld zich verder in de Allergologie te bekwamen. In 1956 volgde zijn inschrijving in het specialisten-register als allergoloog met aantekening Röntgenologie.